Water Quality Assessment and Antibiotic Susceptibility Testing of Bacteria Isolated from Borehole Water Supplies within a Residential Environment in Elele, Rivers State, Nigeria

Mbah E. I.* **and Amuchie N. E.**
Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria.
* Corresponding author: mbahedith@yahoo.com

Abstract: Borehole water supplies within residential settings in southern Nigeria are a major source of drinking water for low- and middle-class families. This study was designed to assess the water quality, phenotypic characterization and antibiogram profiling of bacteria isolated from some borehole water supplies within residential environments in Elele, Rivers State, Nigeria, that low and some middle-class families rely on as their source of drinking water. A total of 30 borehole water samples were collected at various geo-referenced points and subjected to physicochemical analysis and bacteriological before antibiotic susceptibility tests using standard methods. Results of the physicochemical analysis revealed that most water quality parameters fell within the safe limits of the World Health Organization (WHO) except for chromium (2·0mg/L) and fluoride (25-100mg/L) which were above their permissible limits of 0·05 and 1·5 mg/L respectively. Predominant pathogenic bacteria; *Escherichia coli* (7, 50·0%), *Klebsiella pneumoniae* ssp *pneumoniae* (2, 14·3 %), and *Enterobacter* spp (5, 35·7 %) experimentally annotated and confirmed by the VITEK® 2 Compact system, were all multidrug-resistant, MDR (100 %), phenotypes. However, this study unveiled the high rate of sensitivity (100%) for *Klebsiella pneumoniae* ssp *pneumoniae* and *Enterobacter* spp to ofloxacin, streptomycin and gentamycin 100 % resistance to meropenem and chloramphenicol. *E. coli* showed varying sensitivity and resistance levels to the test antibiotics. This study therefore, offers insight to the diversity of the predominant MDR bacterial phenotypes in borehole water within the study area and the use of ofloxacin, streptomycin and gentamycin in the treatment of waterborne infections caused by MDR *Klebsiella pneumoniae* ssp *pneumoniae* and *Enterobacter* spp within the study area. Also, regular evaluation of chromium and fluorine levels in these water supplies is needed to encourage the early development of an intervention strategy once detected above the WHO permissible limit.
Key word: Borehole water, WHONET software, multidrug-resistant bacteria.

INTRODUCTION

Borehole water supplies within different residential settings in Southern Nigeria are a major source of drinking water for the low and some middle-class families. These boreholes are mostly privately owned and there exist no adequate monitoring to validate the efficacy of water treatment (example' Sediment filtration, water softening, disinfection, cleaning, iron removal and so on.), frequency of treatment cycles and also, the maintenance of water piping systems that would ensure that water quality parameters attain the drinking water permissible limits of World Health Organization (WHO). Yusuf *et al*. (2014) documented that unsafe drinking water supplies in Nigeria are due to uncoordinated efforts of various federal, state and local agencies. Waterborne diseases like cholera, dysentery and typhoid fever which can spread rapidly within a population are associated with the contamination of drinking water (Mbah *et al*., 2016; Kumar *et al*., 2022). World Health Organization, WHO, and United Nations International Children's Emergency Fund, UNICEF, (2021) reported that 70% of drinking water in Nigeria at the point of consumption is contaminated. Waterborne diseases arising from the consumption of contaminated water are often caused by pathogens like; viruses, protozoa, bacteria and intestinal parasites. Most of these pathogens shed into the groundwater through fecal contamination to initiate diseases like cholera, typhoid fever and diarrhea (Obuekwe *et al*., 2021). It is however a known fact that the burden of waterborne disease is one of the leading causes of mortality worldwide. Approximately 6.3 percent of mortality worldwide occurs as a result of unsafe water consumption, inadequate sanitation and poor hygiene (WHO, 2015; Kumar *et al*., 2022) and out of which 4 percent of this global disease burden could be preventable through the provision of safe water supply, good sanitation and hygiene. In Nigeria, only about 90 million people have access to improved water (Yusuf *et al.,* 2014) and poor access to improved water and sanitation contributes majorly to high mortality and morbidity rates among children under five (UNICEF, 2022). Also, UNICEF (2022) further documented that the consumption of contaminated water and the use of poor sanitary conditions in Nigeria increases the vulnerability of the populace to waterborne diseases which leads to an annual mortality of more than 70,000 children under the age of five. Microbial contamination of borehole water supplies in Southern Nigeria mostly occurs through seepages of environmental waters into the groundwater aquifer, especially with a sharp rise in the groundwater table during the peak wet seasons (Mbah and Okafor, 2021; Unamba *et al*., 2016) resulting to the inundation of the environment and subsequent destruction of some borehole wells. Antimicrobial resistance (AMR) is the leading cause of mortality worldwide and with the highest burden documented in the low-resource settings (Murray et al., 2022). The emergence and distribution of multidrug-resistant (MDR) bacteria within the clinical and environmental compartments is a major global concern to public health. These MDR bacteria are predominantly members of the Enterobacteriaceae (Teklu *et al*., 2019). The increasing rise of antibiotic resistance in the environmental compartments have been linked to high anthropogenic activities (Finley *et al*., 2013; Mbah *et al*., 2016; Gekenidis *et al*., 2018). Drinking water systems are a major source through which MDR bacteria and other waterborne pathogens are disseminated to the populace (Teklu *et al*., 2019; Falodun *et al.*, 2017), through a fecal-oral route. MDR bacteria such as *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Enterococcus* spp etc. isolated from borehole water supplies have been well documented (Odonkor *et al*., 2022; Obuekwe *et al.*, 2021; Mbah and Okafor, 2021). Globally, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumonia, Acinetobacter baumanii and Pseudomonas aeruginosa* are the six leading MDR bacteria responsible for 929,000 deaths which were attributable to antimicrobial resistance (AMR) and 3.57 million deaths associated with AMR in 2019 (Murray *et al*., 2022). Thus, many researchers are now screening different classes of antibiotics and other good-quality antimicrobials on emerging clinical and environmental isolates of MDR bacteria so as to queue into their national action plan in overcoming this challenge. Following the World Health Organization (WHO) recommendations on the use of AWaRe (Access, Watch, Reserve) classification tool (WHO, 2019) in tackling antimicrobial resistance in achieving antimicrobial stewardship, there is an urgent need to continue evaluating the efficacy of all Watch group antibiotics on all emerging MDR bacteria showing resistance to the Access group antibiotics before further screening with other essential medicines in the Reserved group. In Rivers State, studies on residential borehole water supplies that low- and middle-class families rely on as their sole source of drinking water are limited. It has been ascertained that borehole water supplies at Elele, Rivers State, are emerging hotspots for MDR bacteria (Mbah and Okafor, 2021). However, little is known about the effectiveness of antibiotics on these superbugs within the study area. Thus, this study aimed at investigating the drinking water quality and the sensitivity of isolated MDR bacteria from residential borehole water supplies at Madonna University, Elele against commonly used antibiotics.

MATERIALS AND METHODS

Study sites: The sampling sites selected for this study are located in Madonna University, Elele, and its environs (Figure 1) which is situated in Rivers State. The ten (10) sampling sites used in this study were geo-referenced as follows:

SM (5°08'23.1"N, 6°49'46.2"E);

EH (5°08'13.9"N, 6°49'43.9"E);
FI (5°08'11.0"N, 6°49'39.2"E);
MI (5°08'04.5"N, 6°49'29.9"E);
SC (5°07'57.8"N, 6°49'37.7"E);
EQ (5°08'02.7"N, 6°49'46.5"E);
RC (5°08'09.6"N, 6°49'40.0"E);
BF (5°08'08.0"N, 6°49'41.3"E);
JS (5°08'01.6"N, 6°49'42.0"E);
FL (5°08'27.0"N, 6°49'36.7"E).

This residential environment harbor a good mix of the low, middle and high-income families working within the University community and its environs.

Sample collection: A volume of 1000 mL of borehole water samples from the ten different sites were collected aseptically with sterile screw capped plastic sample containers of 1000 mL capacity. Before each water collection, cotton wool soaked with 70% (v/v) ethanol was used to sterilize the nozzles of the borehole taps to ensure no contamination entered the water (Azuonwu, *et al.*, 2020). Once the taps were turned on, water was allowed to flow for about 2-3 minutes before collection into the well labeled sterile containers and then, transported to the laboratory in an ice packed container. All water samples were processed within six (6) hours of collection in the Department of Microbiology, Madonna University Elele Nigeria.

Physicochemical analyses: On-site physicochemical analyses of the water samples were conducted using both probes (Hanner instrument, USA) and paper-based sensors. The physicochemical analysis that was carried out included; pH, temperature, electron conductivity and total dissolved solids while total alkalinity, hardness, lead, copper, iron, mercury, chromium, bromine, residual chlorine, fluoride, sulfite, nitrate, and nitrite were determined using paper-based biosensors. (Dhanaji *et al*., 2016; Busa *et al*., 2016).

Bacteriological analysis of water: The total cultivable heterotrophic bacterial counts were evaluated by the use of standard methods of pour plating using nutrient agar medium (Enaigbe *et al.,* 2019). The plates were incubated at 37^{o}C for 24 h and distinct colonies were used to calculate the cultivable bacterial load (colony forming units per ml, cfu/mL). Pathogenic bacteria were also isolated from all drinking water samples collected by employing the membrane filtration technique. Approximately 100 ml of each water samples were filtered through 0.45 µm pore size, 47 mm diameter sterile membrane filter (Millipore, Billerica, MA, USA). The membrane filters were then inoculated on solidified Eosin Methylene Blue (EMB) agar media and incubated at two temperatures (37^{o}C and 44.5^{o}C) for 18-24 h. The temperature at 44.5^{o}C allowed for the selection of all *E. coli* present in the water samples. All presumptive colonies were picked, sub-cultured on EMB and characterized by experimental annotation (gram staining, morphological and biochemical characteristics) and VITEK® 2 Compact system, (version 9.02). The use of VITEK® 2 Compact system for the confirmation of all experimentally annotated phenotypes were done at the Medical Laboratory Unit, Calabar Teaching Hospital, Calabar, Cross River, Nigeria.

Antibiotic susceptibility tests of bacterial isolates: Antibiotic susceptibility test of bacterial isolates was carried out using the Kirby–Bauer disc diffusion technique as described by Wakil and Mbah (2012) and Mbah and Okafor (2021). Nitrofurantion (100 µg), Ciprofloxacin (10 µg), Chloramphenicol (10 µg), Gentamicin (10 µg), Ofloxacin (10 µg), Meropenem (10 µg), Pefloxacin (10 µg), Ceftriaxone (30 µg), Amoxicillin (30 µg), and Streptomycin (10 µg); (Oxoid, UK) were used for this test. This was done by introducing about 3-5 well isolated pure young colonies of 18-24 h into normal physiological saline and homogenized to create a bacterial suspension. The turbidity of the various bacterial suspensions was adjusted to the turbidity of 0.5 McFarland standard against a contrasting black and white background and the bacterial load (1-2×10^{8} cfu/mL) was confirmed using a spectrophotometer of 1 cm light path at 625 nm to obtain an absorbance reading of 0.08-0.13. The bacterial suspensions were then inoculated uniformly unto the surface of Mueller Hinton agar plates using sterile swab sticks immersed in the suspension and pressed against the side of the test tubes to remove excess fluid. The antibiotic discs were carefully placed on the dry surface of inoculated plates with the aid of sterile forceps and incubated at 37˚C for 18-24 h. Zones of inhibition were measured in millimeters and mean zones of inhibition recorded were interpreted as susceptible (s), intermediate (I) or resistant (R) following the guidelines of the Clinical and Laboratory Standards Institute (2021). Isolates that were resistant to three or more antimicrobial classes were regarded as MDR.

Statistical analysis: The data for physicochemical parameters of the borehole water supplies within the residential environment were summarized in means ±standard deviation. Distribution of pathogenic bacteria supplies were analyzed in percentages. WHONET data analysis and interpretation were conducted on datasets generated from the antimicrobial susceptibility tests.

RESULTS

Physicochemical and bacteriological analysis of borehole water supplies

The findings obtained from the water quality analysis conducted revealed that all physicochemical parameters including the total cultivable heterotrophic bacteria (TCHB) fell within the permissible limit of World Health Organization (Table 1) except for chromium (2.0 mg/L) and fluoride (25-100 mg/L) across all study sites. A total of 14 pathogenic bacteria were isolated and identified by both experimental annotations and VITEK® 2 Compact systems. From the results, *Escherichia coli* (7, 50.0%), *Klebsiella pneumoniae* ssp *pneumoniae* (2, 14.3%), and *Enterobacter* spp (5, 35.7%) were the prevalent pathogenic bacteria found and with *Escherichia coli* as the most dominant bacteria. However, *Enterobacter cloacae* ssp. *cloacae*, *Enterobacter cloacae* ssp. *dissolvens* and *Enterobacter aerogenes* were detected as the diverse species of Enterobacter in the water supplies (Figure 2).

Antibiotic susceptibility tests of the bacterial isolates

The WHONET-19 analysis of the antimicrobial resistance profiles of bacterial pathogens isolated from the borehole water supplies meant for human consumption within the residential area categorized all tested bacterial pathogens presented in this study as medium priority pathogens. However, due to their high level of resistance to carbapenem, WHONET-15 classified them as high priority pathogens. The antibiotic susceptibility profiles of members of the Enterobacteriaceae involved in this study presented the percentage of isolates that are resistant (including a 95 % confidence interval), intermediate, and susceptible in Fig. 4 (a-d). Out of the ten antibiotics (AMX, MEM, STR, PEF, CIP, CRO, CHL, GEN, OFX and NIT) used for the study; all tested bacterial pathogens were found to be resistant to more than three antibiotics. All isolates that were resistant to at least 3 of the 10 antibiotics categories tested were grouped as multidrug resistant (MDR) phenotypes. In this study, all isolates that their sensitivity to antibiotics was in the intermediate category were also regarded as resistant phenotypes. However, the sensitivity of these MDR bacteria to some antibiotics was also observed. ofloxacin and streptomycin had the highest susceptibility rate for *Klebsiella pneumoniae* ssp *pneumoniae* (100 %), while it was ofloxacin, streptomycin and gentamycin for *Enterobacter aerogenes* (100 %), ofloxacin for *Enterobacter clocae* (100 %) and gentamycin (85.7 %), streptomycin (71.4 %) and ofloxacin (71.4 %) for *E. coli* respectively. *Klebsiella pneumoniae* ssp *pneumoniae* demonstrated 100% resistance to meropenem and chloramphenicol while *Enterobacter aerogenes* showed 100% resistance to meropenem. In addition, *Enterobacter clocae* showed 100% resistance to chloramphenicol while *E. coli* isolates were 85.7% resistant to meropenem and nitrofurantoin respectively.

Figure. 1. A Map of the geo-referenced points within the study location. NB: 1. St. Mathias Lodge, SM, (5°08'23.1"N, 6°49'46.2"E); 2. Engine house, EH, (5°08'13.9"N, 6°49'43.9"E); 3.Female Internship Lodge, FI, (5°08'11.0"N, 6°49'39.2"E); 4. Male Internship Lodge, MI, (5°08'04.5"N, 6°49'29.9"E); 5. Special Canteen Lodge, SC, (5°07'57.8"N, 6°49'37.7"E); 6. European Quarters, EQ, (5°08'02.7"N, 6°49'46.5"E); 7. Reconciliation Lodge, RC, (5°08'09.6"N, 6°49'40.0"E); 8. Benefactor Lodge, BF, (5°08'08.0"N, 6°49'41.3"E); 9. Jesus the Savior Lodge, JS, (5°08'01.6"N, 6°49'42.0"E); 10. Factory Lodge, FL, (5°08'27.0"N, 6°49'36.7"E)

Table 1: Physicochemical and bacteriological analysis of borehole water supplies

Parameters	Unit	SM	FI	JS	SC	EH	EQ	BF	MI	RC	FL	WHO/NSDWQ Limit (2017)
Temp.	° C	26±02	24±02	25±2.5	25±2.5	23±01	25±2.5	25±2.5	24±02	24±02	28.±02	Ambient
P^H		7.3±07	7.0±06	7.6±0.7	7.0±06	7.0±06	7.2±0.7	7.3±07	7.1±06	7.2± 0.7	7.4±0.	6.5-8.5
EC	(S/cm)	26±02	28±2.8	8.0±.0.8	16±1.6	26±2.6	27±2.7	26±2.6	18±1.7	28±2.8	36±3.6	1000
TDS	mg/L	13±1.3	12±1.2	4.0±0.4	9.0±0.9	12±1.2	14±1.4	13±1.3	08±0.8	14±1.4	20±02	500
T. alkalinity	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	-
Hardness	mg/L	50±5.0	50±5.050•0	125±12	50±5•0	125±12	0.0	50±5.0	50±5.0	50±5.0	50±5.0	150
Iron	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0.1
Mercury	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0.006
Chromium	mg/L	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	02±0.2	0·05
Bromine	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0.05
Nitrate	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	50
Nitrite	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	3.0
Cl	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	5.0
Fluoride	mg/L	100±10	25±2.5	100±10	50±5.0	100±10	50±5.0	100±10	50±5.0	50±5.0	25±2.5	1.5
					50•0		50•0		50•0			
Sulfate	mg/L	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	50
TCHB	Cfu/mL	18 $X10^{-1}$	4·0X10	2·2X 10^{1}	1·2X1 0^{1}	2·5X1 0^{0}	1·2X1 0^{0}	1·1.X1 0^{0}	6·0X1 0^{-1}	1·5X1 0^{0}	2·3X1 0^{0}	400

Data are presented in mean ±Standard deviation. SM: St. Mathias Lodge; FI: Female internship Lodge; JT: Jesus the Saviors Lodge; SC: Special canteen Lodge; EH: Engine house; EQ: European Quarters; BF: Benefactors Lodge; MI: Male internship Lodge: RC: Reconciliation Centre Lodge; FL: Factory Lodge; TCHB: Total Cultivable Heterotrophic Bacteria.

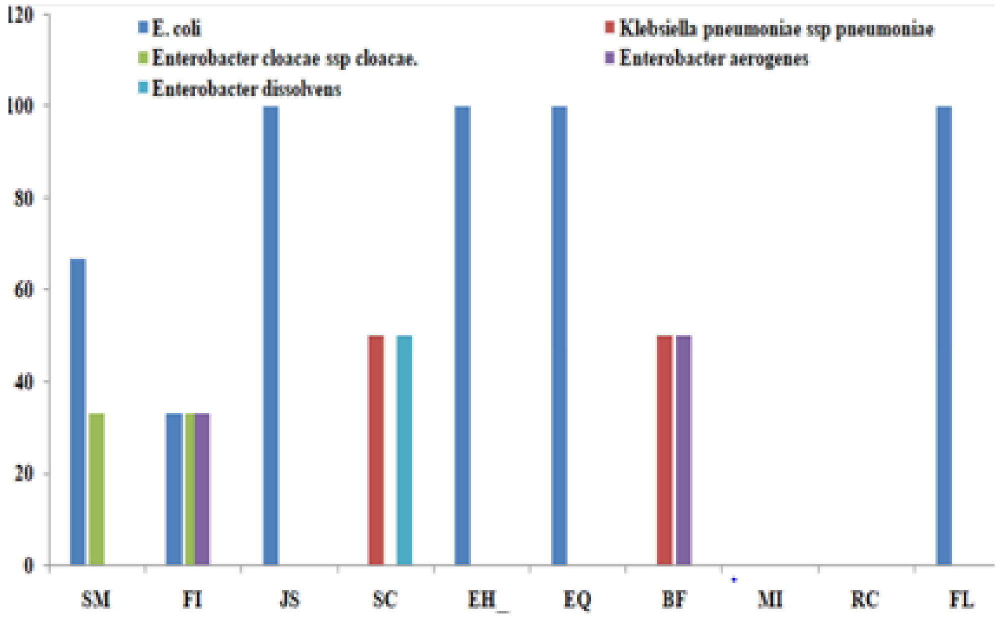

Figure 2. Distribution and relative abundances of pathogenic bacteria in borehole water supplies within the residential area. Key: SM: St. Mathias Lodge; FI: Female internship Lodge; JT: Jesus the Saviors Lodge; SC: Special canteen Lodge; EH: Engine house Lodge; EQ: European Quarters; BF: Benefactors Lodge; MI: Male internship Lodge: RC: Reconciliation Centre Lodge; FL: Factory Lodge

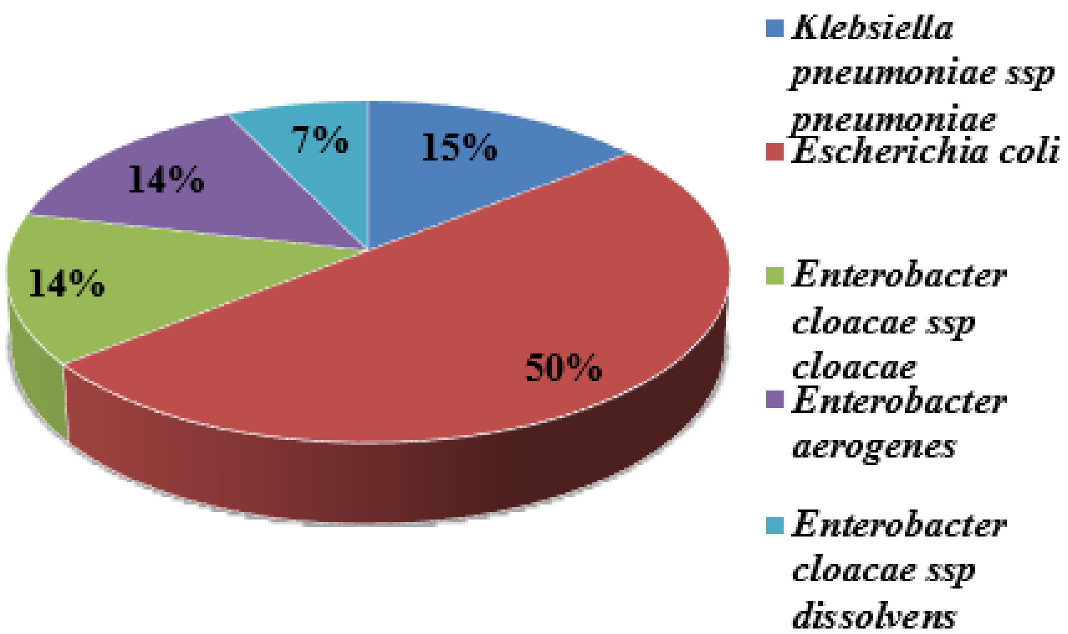

Figure 3: Percentage abundances of pathogenic bacteria recovered from all the borehole water supplies.

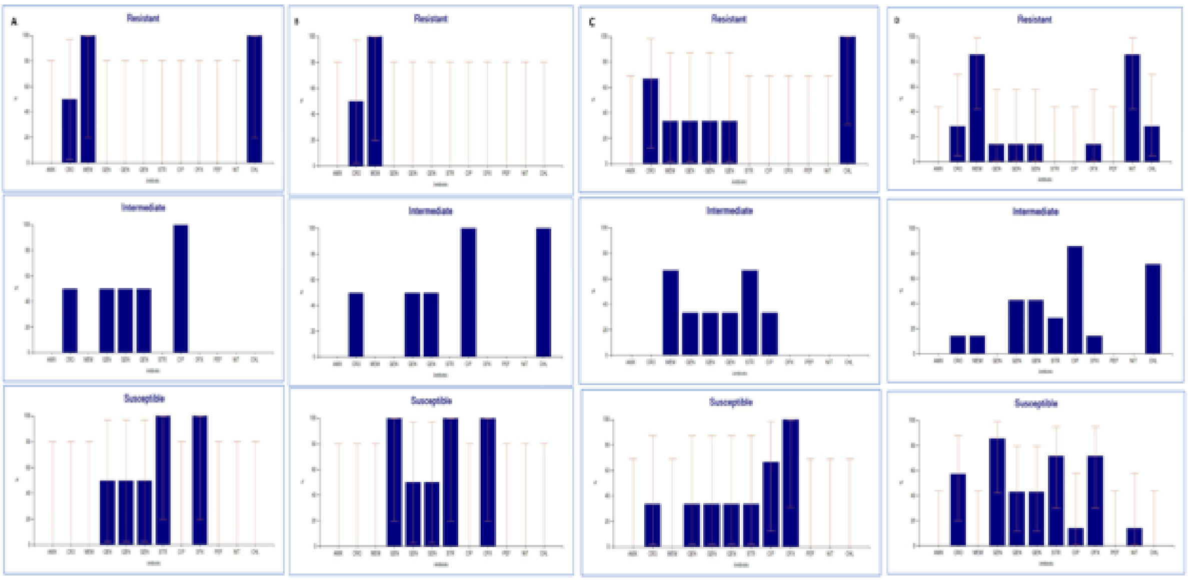

Figure 4. Antibiotic resistant patterns of *Klebsiella pneumoniae* ssp *pneumoniae* (A), *Enterobacter aerogenes* (B), *Enterobacter clocae* (C), and *E. coli* (D) from residential borehole water supplies. Key: Amoxicillin (AMX), Meropenem (MEM), Streptomycin (STR), Pefloxacin (PEF), Ciprofloxacin (CIP),Ceftriaxone (CRO), Chloramphenicol (CHL), Gentamycin (GEN), Ofloxacin (OFX) and Nitrofurantoin

DISCUSSION

This study focused on the water quality assessment and antibiogram profiling of bacteria isolated from some borehole water supplies within residential environments in Elele, Rivers State, Nigeria, that low and some middle-class families rely on as their source of drinking water. This study observed the presence of MDR bacteria and high levels of chromium (2.0 mg/L) and fluoride (25-100 mg/L) in the borehole water supplies that serve as a major source of drinking water for low-and middle- class families.

In Nigeria, microbial quality of drinking water is often guided by the WHO (World Health Organization) and NSDWQ (Nigeria Standard for Drinking Water Quality) water quality guidelines. Following these guidelines for drinking water quality (NSDWQ, 2017; WHO, 2017), the results obtained from this study showed that all the borehole drinking water samples obtained within the residential area of Madonna University, Elele and its environs, had the mean concentration values of the tested parameters fall within the safe limits of WHO and NSDWQ except for chromium (2.0 mg/L) and fluoride (25-100 mg/L) which were above their permissible limits of 0.05 and 1.5 mg/L for chromium and fluorides respectively. Similar results on high floride concentration above the WHO permissible limit have been described by other authors (Ram *et al*. 2017, Dongzagla *et al*. 2019; Egor and Birungi, 2019 & Giwa *et al.* 2021) on studies done in borehole drinking water situated in different residential environments. Drinking water is a major source of fluoride intake to the human body (Dongzagla *et al.* 2019). Fluoride is an element that is very important to the human health; however, its high accumulation in the body may lead to Fluorosis (Waziri *et al*. 2012). The risk of population exposure to high fluoride is largely due to the consumption of large volumes of water during the hot weather (Dongzagla *et al*. 2019). The occurrence of severe dental and skeletal fluorosis has been documented by Giwa *et al*. (2012) in four communities within the North-eastern states of Nigeria.

For the high level of chromium concentrations (2.0 mg/L) above the WHO limit observed from all the tested borehole water samples used in this study, a similar observation of high chromium concentrations in borehole and well water samples in Mgbuoshimiri community in Rivers State have been previously documented by Odu *et al*. (2020).

This study characterized 14 pathogenic bacteria isolated from 30 borehole water supplies within residential environments in Elele, Rivers State of Nigeria by the use of phenotypic methods. *Escherichia coli* (7, 50.0%), *Klebsiella pneumoniae* ssp *pneumoniae* (2, 14.3 %), and *Enterobacter* spp (5, 35.7 %) were the diverse Gram-negative bacteria found and with *Escherichia coli* as the most dominant phenotypes. The occurrence of these Gram-negative bacteria in the borehole water supplies suggests the degradation and fecal pollution of the drinking water supplies within this residential area in Elele community. According to Mbah *et al*. (2016), exposure to drinking water contaminants and its resultant health effect is a major concern to public health. Previous reports have shown that the ingestion of fecal contaminated water may trigger waterborne diseases like, gastroenteritis (Folorunso *et al.,* 2014; Mbah *et al*., 2016)

This study found out that all members of the Enterobacteriaceae isolated were of the multidrug resistant phenotypes. Resistance to different antibiotics can contribute to the existence of newly emerging multidrug-resistant bacteria which may get transmitted to different individuals, causing illnesses that may be difficult to treat (Ayandiran *et al.,* 2014). However, despite the level of antibiotics resistance demonstrated by most bacterial isolates, some bacterial phenotypes were found to show susceptibility to some antibiotics used in this study. Ofloxacin and streptomycin had the highest susceptibility rate for *Klebsiella pneumoniae* ssp *pneumoniae* (100%), while it was ofloxacin, streptomycin and gentamycin for *Enterobacter aerogenes* (100%), ofloxacin for *Enterobacter cloacae* (100%) and Gentamycin (85.7%), ofloxacin and streptomycin (71.4%) for *E. coli*. The bactericidal activity of ofloxacin, streptomycin and gentamycin on Gram negative bacteria contaminating drinking water resources in Nigeria is of great interest to public health. Similarly, to the effectiveness of ofloxacin to most of our bacterial phenotypes, Eddeh-Adjugah *et al*. (2022) documented that amongst all pathogenic *E. coli* isolated from several borehole water taps in Port-Harcourt, Rivers State, ofloxacin showed the highest susceptibility rate of 100 %. Olorunleke *et al.* (2022) also revealed that ofloxacin had the highest susceptibility rate for all *E. coli* isolates recovered from livestock and in-contact human in Southeastern Nigeria.

CONCLUSION

Consumption of borehole water supplies by low- and middle-class families within the residential settings in Elele community in Rivers State, Nigeria should be discouraged due to the occurrence of MDR bacteria and high levels of chromium (2.0 mg/L) and fluoride (25-100 mg/L) above the WHO limits for safe water quality that have been found in this study. Awareness campaigns on high levels of chromium and fluoride above the WHO permissible limits for safe water quality in Elele community is highly recommended. This would encourage families to screen their borehole water supplies for the presence of high levels of chromium and fluorides and then seek for intervention strategies to help reduce their levels.

Acknowledgement

The authors are thankful to the Medical Laboratory Unit, Calabar Teaching Hospital, Calabar, Cross River, Nigeria, for their collaboration during the confirmation of our suspected bacterial phenotypes using the VITEK® 2 Compact system.

REFERENCES

Ayandiran, T.A., Ayandele, A.A., Dahunsi, S.O. & Ajala, O.O. (2014). Microbial assessment and prevalence of antibiotic resistance in polluted Oluwa River, Nigeria. *Egyptian Journal of Aquatic Research,* 40 (3), 291-299.

Azuonwu O, Ihua N. & Ohwondah G. (2020). Bacteriological risk assessment of borehole sources of drinking water in some part of Port Harcourt Metropolis of Niger Delta, Nigeria. *Biomedical Journal of Scientific and Technical Research.* 24 (4), 18477-18487.

Busa, L.S.A., Mohammadi, S., Maeki, M., Ishida, A., Tani, H., & Tokeshi, M. (2016). Advances in microfluidic paper-based analytical devices for food and water analysis. *Micromachines*, 7(5), 86.

Dhanaji, K.G., Shagufta, S.A. & Pramod, J.N. (2016). Physico-Chemical Analysis of Drinking Water Samples of Different Places in Kadegaon Tahsil, Maharashtra (India), Pelagia Research Library. *Advances in Applied Science Research*, 7(6), 41-44.

Dongzagla, A., Jewitt, S. & O'Hara, S. (2019). Assessment of fluoride concentrations in drinking water sources in the Jirapa and Kassena-Nankana Municipalities of Ghana. https://doi.org/10.1016/j.gsd.2019.100272.

Eddeh-Adjugah, O., Ogbonna, D., Sabastine, N. & Youdeowei, P. (2022). Antibiogram Pattern of Bacteria Isolates from Ground Water (Borehole Water) Resources in Port Harcourt, Southern Nigeria. *Asian Journal of Environment and Ecology;* 19-32. 10.9734/ajee/2022/v18i330317.

Enaigbe, A.A., Ekhaise, F.O., Idemudia, I.B., & Akpoka, A.O.J. (2019) Physicochemical and Microbiological Analyses of Bacterial Isolates from Drinking Water Distribution Systems of Some Higher Institutions in Edo State, Nigeria. *Applied Science Environmental Management.* 23(5), 909-915.

Falodun, I.O., Morakinyo, M.Y. & Fagade, E.O. (2017). Determination of Water Quality and Detection of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Selected Rivers Located in Ibadan, Nigeria. *Jordan Journal of Biological Sciences*, 11(1995-6673), 107 – 111.

Finley, R. L., Collignon, P., Larsson, D. G., McEwen. S. A., Li, X. Z., Gaze, W. H., Reid-Smith, R., Timinouni, M., Graham, D. W., & Topp, E. (2013). The scourge of antibiotic resistance: the important role of the environment. *Clinical Infectious Disease,* 57(5), 704-10. https://doi.org/10.1093/cid/cit355.

Gekenidis, M.-T., Qi, W., Hummerjohann, J., Zbinden, R., Walsh, F., & Drissner, D. (2018). Antibiotic-resistant Indicator Bacteria in Irrigation Water: High Prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli*. *PLoS One.* 13, e0207857. doi: 10.1371/JOURNAL.PONE.0207857.

Giwa, A., Crutchfield, D., Fletcher, D., Gemmill, J., Kindrat, J., Smith, A., & Bayless, P. (2021). Addressing Moral Injury in Emergency Medicine. *Journal Emergency Medicine,* 61(6), 782-788. https://doi.org/10.1016/j.jemermed.2021.07.066.

Kumar, P., Srivastava, S., Banerjee, A., & Banerjee (2022). Prevalence and predictors of water-borne diseases among elderly people in India: evidence from Longitudinal Ageing Study in India, 2017-18. *BMC Public Health*, 22:993. https://doi.org/10.1186/s12889-022-13376-6.

Mbah, E. Okafor, C. (2021). Exploring Borehole Drinking Water Supplies for the Occurrence and Prevalence of Extended- Spectrum β-Lactamase- and Carbapenemase- Producing E. coli, *Proceedings of the 2 Biotechnology Society of Nigeria (BSN), South-East Zonal Symposium "Abia 2021"* (2), 205-210.

Mbah, E.I., Abu, O. G., & Ibe, N. S. (2016). A metagenomes-based investigation of the impact of natural run-offs and anthropogenesis on a freshwater ecosystem at points of use in Niger Delta, Nigeria. *International Journal of Innovative Research and Development*, 297-304.

Murray, J. C., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, R. G., Gray, A., Han, C., & Bisignano, C. (2022). Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. 12;399(10325), 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0.

Obuekwe, S.I., Oshoma, C. & Omorogbe, N.J. (2021). Evaluation of Physicochemical and Bacteriological Properties of Borehole Water in Selected Areas of Benin City, Edo State. *Nigerian Journal of Life Science*, 11(1), 10-17. https://doi.org/10.52417/njls.v11i1.59

Odonkor, T.S., Simpson, V.S., Medina, M.R.W. & Fahrenfeld, L N. (2022). Antibiotic-Resistant Bacteria and Resistance Genes in Isolates from Ghanaian Drinking Water Sources. *Journal Environmental Public Health*, 2850165. https://doi.org/10.1155/2022/2850165.

Olorunleke, S.O., Kirchner, M., Duggett, N., Abuoun, M., Okorie-Kanu, O.J., Stevens, K., Card, R.M., Chah, K.F., Nwanta, J.A., Brunton, L.A., & Anjum, M.F. (2022). Molecular characterization of extended spectrum cephalosporin resistant Escherichia coli isolated from livestock and in-contact humans in Southeast Nigeria. *Frontiers of Microbiology*, 13:937968. doi: 10.3389/fmicb.2022.93796.

Teklu, D.S., Negeri, A.A., Legese, M.H., Bedada, T.L., Woldemariam, H.K., & Tullu, K.D. (2019). Extended-spectrum beta-lactamase production and multi-drug resistance among *Enterobacteriaceae* isolated in Addis Ababa, Ethiopia. *Antimicrobial Resistance Infections Control*, 839. https://doi.org/10.1186/s13756-019-0488-4.

Unamba, L.C., Nwachukwu C.E. & Isu, R.N. (2016). Physicochemical and bacteriological assessment of some borehole wters in the Federal Capital Territory, Nigeria. *International Research Journal of Public and Environmental Health;* 3 (6), 140-145; http://dx.doi.org/10.15739/irjpeh.16.018.

Wakil, S.M & Mbah, E. I. (2012). Screening antibiotics for the elimination of bacteria in *in vitro* yam plantlets. *Assumption Journal of Technology*. 16 (1), 7-18.

WHO (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO communicable disease control, prevention, and eradication. WHO pesticide evaluation scheme; 2005. WHO/CDS/WHOPES/GCDPP/2005.13.

WHO (2015). Microplastics in drinking-water https://www.who.int/publications/i/item/9789241516198.

WHO (2017) Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum. https://www.who.int/publications/i/item/9789241549950, WHO (2019). WHO AWaRe Classification Database of Antibiotics for Evaluation and Monitoring of Use. Available online: https://www.who.int/publications/i/item/WHOEMPIAU2019.11

WHO/UNICEF (2021). Progress on household drinking water, sanitation and hygiene 2000-2020: five years into the SDGs. World Health Organization. https://iris.who.int/handle/10665/345081. License: CC BY-NC-SA 3.0 IGO

WHO/UNICEF (2021). Joint Monitoring Program for Water Supply, Sanitation and Hygiene (JMP) - Progress on household drinking water, sanitation and hygiene 2000 – 2020.

Yussuf, A.S., John, W. & Oloruntoba, A.C. (2014). Review on Prevalence of Waterborne Diseases in Nigeria; *Journal of Advancement in Medical and Life Sciences,* 1(2), 1-3.