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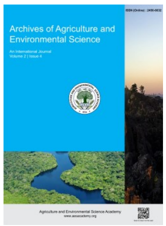

ORIGINAL RESEARCH ARTICLE

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Physicochemical characteristics and antibiotic resistance patterns of enteric bacteria isolated from harvested rainwater (HRW) in Oraukwu, Anambra State, Nigeria

E.C.S. Okoye[1], S.C. Onuorah[2], L.C. Okoye[3], L.C. Eleanya[1], G.C. Agu[4], U.C. Okafor[2] and N.A. Egudu[5]

[1]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State, Nigeria
[2]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[3]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria
[4]Bacterial Vaccine Production, National Veterinary Research Institute, Vom Plateau State, Nigeria
[5]Department of Agricultural Technology and Microbiology, School of Agriculture, Enugu State Polytechnic, Iwollo Enugu State, Nigeria
[*]Corresponding author's E-mail: ec.okoye@coou.edu.ng

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ABSTRACT

The aim of the study was to evaluate the physicochemical properties and antibiotic resistance patterns of enteric bacteria isolated from HRW in Oraukwu, Anambra State. Duplicate water samples were aseptically collected from below-the-ground concrete reservoirs in twelve households using sterile 5 L plastic containers. Physicochemical parameters such as pH, temperature, colour, turbidity, DO, TDS, TSS, alkalinity, hardness, chloride, and some heavy metals of the water samples were examined using APHA method. Bacteriological analyses were performed using the membrane filtration technique. Colonies formed were counted and expressed in CFU/100mL. Enteric bacteria were enumerated and characterized by their morphological characteristics and biochemical tests. Axenic cultures of the isolates were further subjected to antimicrobial susceptibility testing (AST) using the modified Kirby-Bauer disc diffusion method, based on the guidelines by CLSI. Results revealed that physicochemical parameters and some heavy metals were within acceptable limits, except for Fe (0.01-0.72 mg/L) and Pb (0.01-0.25 mg/L). Total bacterial counts ranged from 1.2×10^4 to 6.8×10^4 CFU/100mL, indicating high contamination. Morphological characteristics revealed twelve (12) isolates of enteric bacteria, comprising *Escherichia coli* (41.67%), *Salmonella* sp. (33.33%) and *Shigella* sp. (25%). All isolates exhibited 100% resistance to augmentin and tetracycline, but showed varying degrees of susceptibilities; *E. coli* to levofloxacin (60%), *Salmonella* sp. to ertapenem, imipenem, levofloxacin and nalidixic acid (100%), and *Shigella* sp. to ceftriaxone and ertapenem (100%). 'First flush' diverters are recommended to be installed within the water collecting system, in order to divert runoff from the rooftop after a period of no rainfall. Antibiotics which the isolates were susceptible to are recommended for the treatment of infections caused by these pathogens.

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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 resource that is linked to the survival of man and other living things (Abaasa *et al*., 2024). In emerging nations like Africa, millions of people suffer from water-borne infections (Khayan *et al*., 2019). The WHO posits that 1 of every 5 infant deaths, especially between ages of 1 to 5 in sub-Saharan Africa, is associated with inadequate wash

(WHO, 2019). According to the United Nations Children's Fund (UNICEF), about 4000 children pass away per day globally due to preventable contaminated water causes (UNICEF, 2014). Over one billion people in the world lack access to safe drinking water, and about 2.5 billion people do not have access to adequate sanitation services at all (WHO & UNICEF, 2024). The sixth sustainable development goal (SDG 6) of the United Nations Agenda 2030 - Clean Water and Sanitation for All, states that more than 733 million people still live in countries with high and critical levels of water crisis (UN, 2019). Consequently, various countries have adopted programmes aimed at improving the quality and accessibility of water to their citizens. The paucity of piped-borne water supply in most rural communities and even urban cities necessitated storing rainwater in tanks and underground concrete reservoirs as alternative sources of water supply, used for both drinking and domestic purposes (Nnaji *et al.*, 2018; Zdeb *et al.*, 2020).

Rainwater is obtained from rainfall, and it is one of the forms of surface water. It occurs as a result of the condensation of water molecules in the atmosphere, and could be collected from different catchments (Campisano *et al.*, 2017; Zdeb *et al.*, 2020; Nwogu *et al.*, 2024). Rainfall is readily available during wet seasons and can be used at multiple scales from residential to commercial levels. From the earliest times, rainwater has been, and it is still an excellent source of drinking water, especially for people in rural communities. It is collected, piped into clean and closed underground tanks known as reservoirs or tanks, and used later. However, it can be contaminated by dirty roofs, dirty containers and tanks (Kelemewerk *et al.*, 2020). Harvested rainwater (HRW) is one of the dependable sources of domestic water supply for the residents of Oraukwu, Anambra State, Nigeria. It involves collection and storage of rainwater in a natural or artificial container, either for immediate use or use before the onset of the next season for domestic, agricultural, industrial and environmental purposes (Girma *et al.*, 2019; De Sá Silva *et al.*, 2022; Ross *et al.*, 2022; Ghodsi *et al.*, 2023). Despite its usefulness, HRW stored in underground tanks harbour millions of microbes, resulting from animal wastes and organic matter on the rooftops or land areas (Sefton *et al.*, 2022). The initial water runoff from rooftops usually contains impurities from particles of dust and other harmful substances. HRW can also harbour traces of heavy metals, which can be washed down from roofing materials of the catchment surface, and this may pose severe health risks (Naser *et al.*, 2017; Nnaji *et al.*, 2018). Previous studies on physical and chemical properties of harvested rainwater showed that critical physicochemical parameters were within acceptable limits, using standard guidelines set by the WHO and NIS. Several studies reveled that below-the-ground concrete reservoirs are situated close to septic tanks, refuse dumps and animal farms, and this can lead to microbial contamination with enteric bacteria (Hamilton *et al.*, 2019; Waideman *et al.*, 2020).

The microbiological quality of drinking water is among the prerequisites for the provision of safe and clean water for human consumption (Khan *et al.*, 2023). This is because drinking water contaminated with pathogenic microorganisms exposes an individual to various water-borne diseases such as cholera, typhoid fever, dysentery, and gastroenteritis, which are characterized by diarrhoea (Pal *et al.*, 2018; Amos *et al.*, 2020; Okoye *et al.*, 2022b). Faecal bacteria such as *Escherichia coli*, *Shigella* sp., *Klebsiella* sp., *Salmonella* sp., etc. and other pathogenic bacteria have been isolated from harvested rainwater, stored in tanks and underground reservoirs (Amin & Alazba, 2019; Ewelike *et al.*, 2020; Morka, 2022). The World Health Organization (WHO) guidelines for drinking water quality identify faecal contamination as the greatest risk to human health associated with drinking water quality (Ekelozie *et al.*, 2018).

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 (Reygaert, 2018; Fukuda *et al.*, 2023). This development requires urgent attention by health policy makers 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, but only a few extensive studies exist on the prevalence of antibiotic resistance in harvested rainwater samples from solely residential areas (Khayan *et al.*, 2019; Onah *et al.*, 2019; Maspalma *et al.*, 2022). Hence, the study examined the physicochemical characteristics of harvested rainwater samples obtained from Oraukwu, Anambra State, and profiled the antibiotic resistance patterns of enteric bacteria in the water samples.

MATERIALS AND METHODS

Description of the study area

Oraukwu, a town in Idemili North Local Government Area of Anambra State, Nigeria, is geographically located between latitude 6°06' 31" North and longitude 6°59' 16" East, and has an elevation of 180m above sea level. It is bounded in the East by Abacha and Nimo, West by Adazi Ani and Neni, North by Alor, and South by Abatete. It has a population of 60,700 according to the 2006 national census, and includes adults and children, with a growth rate of 3%. The most dominant source of water in the area is rainwater, followed by streams. This is due to the unavailability of piped-borne water, and also due to the high depth of groundwater level which makes groundwater sourcing uneconomical.

Study design and description of sites

Harvested rainwater (HRW) samples were collected for a period of three months (May-July, 2023), and 12 different households with underground concrete reservoirs were randomly selected in Oraukwu, Anambra State. The examined storage reservoirs are made of concrete walls and a top surface that extends 2 feet from the ground surface level, and is covered with a circular reinforced concrete slab. An opening is provided at one edge for the purpose of water extraction from the storage by manual means, using a fetching bucket which is attached to a rope. The opening is provided with a lid that must be closed when the reservoir is not in use, thereby making the facility safe and well protected from external factors such as sunlight, dust, birds droppings, reptiles and their excreta, etc. which may contaminate the stored water.

Sample collection

Duplicate harvested rainwater samples from selected households in Oraukwu, Anambra State were collected over 3-month sampling regime (May-July, 2023) using sterile 5-L plastic containers. Water samples were collected from underground concrete reservoirs by lowering a sterile 5-Litre containers into the pool of water, and filling them with water. Each of the sample was collected in duplicates, and used for physicochemical and bacteriological analyses. Samples were labelled properly (reservoirs A to L), and transported to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria for bacteriological analysis, while samples for physicochemical analysis were transported to Graceland Analytical Laboratory, Awka Anambra State, Nigeria.

Determination of physicochemical parameters

The pH and temperature of the water samples were determined in-situ, using the APHA (2023) method, and were recorded accurately using a pH meter (Uric 9v) and thermometer (Tex care). Analyses for other physicochemical parameters such as turbidity, colour, taste, total dissolved solids (TDS), total suspended solids (TSS), dissolved oxygen (DO), alkalinity, hardness, chloride, and heavy metals were performed. Concentrations of heavy metals were determined using atomic absorption spectrophotometer (AAS).

Determination of total bacterial counts

The total bacterial count was carried out using the membrane filtration technique, as described by Cheesbrough (2006). Water samples were filtered through 0.45 µm pore size filter paper, using two stages vacuum pump (Ve 215). The filter papers were aseptically placed on nutrient agar plates, to obtain total bacterial counts. Colonies formed were counted using the colony counter, and thereafter sub-cultured on agar plates containing Eosin Methylene Blue (EMB) agar and Salmonella-Shigella agar, and incubated at 37 °C for 24 hours.

Isolation and enumeration of enteric bacteria

Discrete colonies on Eosin Methylene Blue (EMB) agar and Salmonella-Shigella agar were counted and recorded, and thereafter sub-cultured onto nutrient agar. Morphological characteristics (pattern of growth, shapes and appearance/sizes on EMB and SSA agar plates) were observed after incubation. Cell morphology (Gram reactions) and other biochemical tests of the isolates were done. Further identification was made by comparison of their cultural, morphological and physiological characteristics with those of known *taxa* using the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 2002).

Antibiotics sensitivity testing (AST)

The AST of the identified isolates was performed against a wide range of antibiotics, using the Kirby-Bauer disc diffusion method, as modified by Nassar *et al.* (2019). The identified bacterial isolates were standardized to 0.5 MacFarland turbidity prior to antimicrobial susceptibility testing. This was swabbed on the entire surface of solidified Mueller-Hinton agar plates using sterile 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 agar plates were allowed to dry for a few minutes, and sterilized forceps were used to place the antibiotics discs evenly on the inoculated agar plates so that the disc was 15mm from the edge of the plate. After 30 minutes, the plates were inverted and incubated at 37 °C for 24 hours. After incubation, the diameter of the zones of growth inhibition around discs was measured to the nearest millimeter using a meter rule. The results were interpreted as sensitive or resistant using the performance standards of CLSI (CLSI, 2020). Standard antibiotic discs (Table 1) were used against the isolates.

Multiple antibiotic resistance phenotype (MARP) and multiple antibiotic resistance index (MARI)

MARP is the number of antibiotics which a particular isolate is resistant to. MAR index is calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics to which the organism is exposed. MARI is calculated as a/b, where a = the number of antibiotics isolate was resistant to, b = the total number of antibiotics used. Bacteria having MAR index ≥ 0.2 originate from a high-risk source of contamination where several antibiotics are used (Malema *et al*., 2018; Afunwa *et al*., 2020; Manyi-Loh *et al*., 2023).

Quality control

The reliability of the study findings was guaranteed by implementing quality control measures throughout the whole laboratory work. Staining reagents, culture media, and antibiotic discs were checked for their normal shelf life before use. All culture plates and antibiotic discs were stored at recommended refrigeration temperature after being prepared and sterilized by autoclaving at 121 °C for 15 minutes. The standard reference bacterial strains were tested as a positive control on the biochemical tests, and agar plates with antibiotic discs.

Statistical analysis

This was conducted using the SPSS software. One-way analysis of variance (ANOVA) was performed to determine whether there was a significant difference between all locations for each parameter at a confidence level of p-value = 0.05. The Pearson correlation analyses were also done to analyze the relationship between the parameters.

RESULTS AND DISCUSSION

Physicochemical properties

Water for human consumption should be colourless, tasteless, odourless and free of turbidity, and must be free of chemicals and should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution (Khayan *et al*., 2019; Onah *et al*., 2019; Maspalma *et al*., 2022). The results of the examined physicochemical parameters are presented in Table 1. Most parameters were within the acceptable limits by the World Health Organization (WHO) and Nigerian Industrial Standards (NIS). The HRW samples had acceptable levels of pH in the range of 6.48-7.18. This is an acceptable range, as it falls within the WHO and NIS standards. The pH values of water samples analyzed in this study was slightly higher than the mean values obtained from Zaria road, Kano metropolis, as reported by Mukhtar *et al.* (2019), while Onah *et al.* (2019), reported values above the present study from five different sites in Enugu metropolis. This implies that the HRW samples in this study were neither acidic nor alkaline in nature, close to neutrality. The values of temperature obtained ranged between 27.8 to 29.8 °C, with a general mean of 29.0 °C. This is generally classified as *ambient* temperature according to the WHO and NIS. The values agree with the reports of Amin & Alazba (2019) and Nwogu *et al.* (2024), who reported that temperatures of HRW samples ranged between 26.8 to 28.2 °C and 27.2 to 29.5 °C, respectively. However, the values of temperature in this present study contradicts the study of Morka (2022), who reported that temperature in HRW in Eku and Abraka, Delta State, Nigeria, were between 26.0-27.2 °C.

Colour is an important physical quality of water, which affects its acceptability by consumers. The values of colour in this study ranged between 5.0 to 8.4 TCU, which is within the acceptable limit of 10 TCU, as recommended by WHO and NIS. Therefore, the colour of HRW samples is considered as 'colourless'. This was also reported by Onah *et al*. (2019), although Coal camp and Agbani road samples recorded higher values of colour. He attributed it to the mineral composition of the sites, which could affect the colour of the water especially if iron compounds are present. Hardness and alkalinity of drinking water are said to be acceptable at 150 mg/L and 100 mg/L respectively, according to the WHO and NIS. Based on these standards, the levels of total hardness and alkalinity recorded for all the samples can be said to be within safe limits. Other researchers also corroborated the levels of alkalinity and hardness in HRW samples (Morka, 2022; Teston *et al*., 2022; Fukuda *et al*., 2023; Nwogu *et al*., 2024). The levels of TDS and TSS are related to one another (Mazurkiewicz *et al*., 2022), and it can be observed that the TDS of the samples increased geometrically with increasing TSS as presented in Table 1. Also, the levels of TDS (10.5 – 135 mg/L) and TSS (45.82- 224.5 mg/L) of all HRW samples were found to be within the standard limit of 500 mg/L as recommended by WHO (2008) and NIS (2007).

In the case of turbidity, the limit is 5 NTU. Most of the analyzed samples had turbidity level below 5 NTU, except for sites D and J which levels (9.58 NTU) and (6.52 NTU) respectively, which had levels above the standard limit. In most waters, turbidity is due to colloidal and extremely fine dispersions (Girma *et al*., 2019; Słyś & Stec, 2020; Ghodsi *et al*., 2023). The traces of heavy metals in HRW are also shown in Table 2. The concentration of Pb were found to be between 0.01 to 0.25 mg/L, and this is slightly above the standard limit of 0.001. This could be attributed to presence of objects such as radio and phone batteries, pencil, erasers, paints, e.tc, in the reservoir tanks. These materials are made of Pb substances, and can found its way into underground reservoirs by playful acts from children or leaching of plastered walls of the storage reservoirs. Amin & Alazba (2019), also reported that Pb is one of the probable sources of rainwater contamination. Similarly, Onah *et al.* (2019), reported elevated levels of Pb in HRW samples of Enugu metropolis, Nigeria. High levels of lead in drinking water can be hazardous to health, as it can linked to lead poisoning. The concentrations of Fe were found to be in the range of 0.01 to 0.72 mg/L, and most of the HRW samples exceeded WHO limits of 0.3 mg/L for drinking water. Mukhtar *et al.* (2019), reported that three out of seven HRW samples analyzed had Fe levels above WHO and NIS limits. Iron is not hazardous to health, but it is considered a secondary or aesthetic contaminant. Essential for good health, iron helps transport oxygen in the blood (IDPH, 1999). Concentrations of Cu range from 0.01 to 1.02 mg/L, and it was observed that all the HRW samples had Cu within the WHO limit of 1.0 mg/L, except at site L which had 1.02 mg/L. Ewelike *et al.* (2020) and Okoye *et al.* (2022a), reported that Cu levels in HRW samples in Southeast and spring water sources in Oji River LGA, Enugu State, Nigeria respectively were within the WHO limits. Anabtawi *et al.* (2022), attributed presence of trace metals in HRW to anthropogenic activities such as indiscriminate dropping of materials or substances made of heavy metals into the reservoir, and also from rusting of metal lids of the reservoirs.

Table 1. Details of the antibiotics that were used in the study to test for antibiotic resistance (CLSI, 2020).

Group	Antibiotics	Abbreviation	Disc potency (μg)	Zone of Resistant <or = nm	inhibition Intermediate nm	Susceptible = or > nm
Combined β-lactam	Ampiclox	ACX	30	≤ 13	14-22	≥ 23
	Augmentin	AUG	30	≤ 13	14-17	≥ 18
Carbapenem	Ertapenem	ERT	10	≤ 18	19-21	≥ 22
	Imipenem	IMP	10	≤ 19	20-22	≥ 23
	Nalidixic acid	NA	30	≤ 13	14-18	≥ 19
Fluoroquinolones	Ciprofloxacin	CIP	5	≤ 20	21-30	≥ 31
	Levofloxacin	LBC	5	≤ 16	17-20	≥ 21
Tetracyclines	Doxcycline	DXT	30	≤ 10	11-13	≥ 14
	Tetracycline	TET	30	≤ 11	12-14	≥ 15
Cephalosporins	Cefuroxime	CXM	30	≤ 14	15-22	≥ 23
	Ceftriaxone	CRO	30	≤		≥
Folate	Trimethoprim-sulfamethoxazole	TMP-SMX	25	≤ 10	11-15	≥ 16

Source: The concentrations used as well as measurement of zones of inhibition were according to the National Committee on Clinical Laboratory Standards Institute (CLSI, 2020).

Table 2. Physicochemical parameters in harvested rainwater samples in Oraukwu, Nigeria.

Parameter	A	B	C	D	E	F	G	H	I	J	K	L	WHO	NIS
pH	6.48	6.86	7.02	7.18	7.01	7.07	6.71	7.10	7.06	6.91	6.95	7.13	6.5-8.5	6.5-8.5
Temp (°C)	29.8	29.7	29.3	29.8	28.5	29.6	28.0	28.5	29.5	29.8	27.8	28.1	Ambient	Ambient
Tur (NTU)	3.06	3.42	3.85	9.58	4.18	3.17	3.68	3.2	3.85	6.52	4.02	3.81	5	5
Colour	5.0	7.5	5.5	8.4	6.5	5.0	6.2	6.4	8.2	7.6	7.4	5.8	10	10
TDS (mg/L	10.5	25.0	38.0	135	83.7	15.0	45.0	54.5	101.5	108.0	30.8	35.8	500	500
TSS (mg/L	45.82	80.05	92.5	224.5	145.8	55.87	85.45	120.5	190.5	215.23	63.58	90.50	500	500
DO (mg/L)	12.0	14.5	11.6	12.8	12.3	10.8	12.4	11.8	12.2	10.5	11.5	10.5	5	5
Alk (mg/L)	7.2	15.2	6.5	30.5	12.8	10.5	14.8	15.5	8.5	21.5	17.3	14.5	100	100
Had (mg/L)	8.5	18.4	22.4	38.4	32.0	16.8	28.7	6.50	19.2	27.5	22.8	15.5	150	150
Cl (mg/L)	5.64	15.18	20.51	28.60	18.65	19.70	4.48	18.92	13.48	14.52	12.51	22.65	250	250
Pb (mg/L)	0.05	0.08	0.05	0.15	0.02	0.06	0.03	0.04	0.01	0.02	0.01	0.25	0.01	0.01
Cu (mg/L)	0.02	0.01	0.03	0.85	0.06	0.03	0.08	0.07	0.04	0.65	0.02	1.02	1	1
Fe (mg/L)	0.03	0.30	0.48	0.56	0.40	0.45	0.35	0.38	0.52	0.50	0.01	0.72	<0.30	<0.30

Key: Temp=Temperature; Tur=Turbidity; TDS=Total dissolved solids; TSS=Total suspended solid; DO=Dissolved oxygen; Alk=Alkalinity; Had=Hardness; Cl=Chloride; Pb=Lead; Cu=Copper; Fe=Iron; NIS = Nigerian Industrial Standards; WHO = World Health Organization.

Table 3. Evaluation of total bacterial counts in harvested rainwater samples in Oraukwu.

Sites	A	B	C	D	E	F	G	H	I	J	K	L
CFU/mL	2.4×10^4	1.2×10^4	1.8×10^4	4.8×10^4	3.7×10^4	5.1×10^4	2.8×10^4	5.8×10^4	6.3×10^4	6.2×10^4	6.8×10^4	3.2×10^4

Table 4. Colonial morphological, microscopic and biochemical characteristics of the bacterial isolates from HRW.

Cell morphology	Colour	Gram rxn	MOT	CAT	OX	IND	MR	VP	CIT	Detected organism
Rod	Metallic green sheen	-	+	+	-	+	+	-	-	*Escherichia coli*
Rod	Colourless	-	-	+	-	-	+	+	+	*Salmonella* sp.
Rod	Opaque	-	+	+	-	-	+	-	-	*Shigella* sp.

Key: + = positive, - = negative, Gram rxn = Gram reaction, MOT = Motility test, CAT = Catalase test, OX = Oxidase test, IND = Indole test, MR = Methyl red test, VP = Voges-Proskauer, CIT = Citrate test.

Enumeration of bacteriological parameters

Total bacterial counts

Total bacterial counts obtained from this study ranged from 1.2×10^4 to 6.8×10^4 CFU/100mL (Table 3), showing a high level of contamination. These values are above the guidelines set by the World Health Organization (WHO, 2008) and Nigerian Industrial Standards (NIS, 2007), which recommend that total heterotrophic bacterial count in 100 mL drinking water should be <100, as counts above 100 CFU/100 mL indicate a general decrease in water quality. Hence, the HRW samples used by Oraukwu residents are highly contaminated, and this poses a risk to those consuming the water. The results agreed with the findings of Mazurkiewicz *et al*. (2022), who reported that total heterotrophic bacterial counts in HRW, stored in underground retention tanks in Poland ranged from 1.9×10^4 to 2.64×10^5 CFU/100 mL. Several other researchers reported similar results of high values of total bacterial counts in HRW (Khayan *et al*., 2019; Ewelike *et al*., 2020; Tiwari *et al*., 2021; Maspalma *et al*., 2022). This study has shown that HRW 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 WHO and NIS standards for coliform bacteria of zero total coliform per 100 mL of drinking water. Ghodsi *et al*. (2023), opined that siting of reservoirs 20-30m away from septic tanks will prevent sewer flow into nearby reservoirs, and this would ensure zero faecal coliform in the water source.

Morphological and biochemical identification of bacterial isolates

Morphological and biochemical tests revealed the presence of different rod-shaped bacteria in HRW samples (Table 4), and twelve (12) isolates were recovered; *Escherichia coli* (n=5), *Salmonella* sp. (n=4) and *Shigella* sp. (n=3). *Escherichia coli* was the most prevalent isolate (41.7%) in HRW samples, with *Salmonella* sp. having 33.3% and *Shigella* sp. having 25%. Nwogu *et al.* (2024), reported presence of *E. coli*, *Shigella dysentriae*, *Enterococcus faecalis* and *Salmonella typhi* in the harvested rainwater from rooftops in Umuahia, Southeastern Nigeria. It is widely acknowledged that the presence of *E. coli* in a drinking water sample is an indication of faecal contamination, and the potential presence of other pathogenic organisms. There should be 0% CFU/100 mL of enterococci and *E. coli* counts in drinking water in the order of 0 CFU/100 mL (conformity), 1–10 CFU/100 mL (low risk), 10–100 CFU/100 mL (intermediate risk), 100–1000 CFU/100 mL (high risk), and above 1000 CFU/100 mL (very high risk) (Gwimbi *et al*., 2019). The presence of *E. coli* in drinking water, therefore, implies the water in unfit for drinking. The presence of *Salmonella* sp. and *Shigella* sp. as reported in this study could lead to gastroenteritis. The result is consistent with the finding of Ekelozie *et al*. (2018), who reported human health risks associated with drinking water contaminated with *Salmonella* sp. in Anambra State, Nigeria. Olalemi *et al*. (2021) and Nwogu *et al*. (2024) also reported the presence of these pathogenic bacteria (*Salmonella* sp. and *Shigella* sp.) in harvested rainwater in Ogun State and Abia Sate, Nigeria respectively.

Table 5. Antibiotic resistance patterns of enteric bacterial isolates in harvested rainwater samples.

Antibiotics	*E. coli* (n=5)			*Salmonella* sp. (n=4)			*Shigella* sp. (n=3)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
ACX	5 (100)	0 (0)	0 (0)	3 (75)	1 (25)	0 (0)	3 (100)	0 (0)	0 (0)
AUG	5 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
CIP	2 (40)	1 (20)	2 (40)	4 (100)	0 (0)	0 (0)	1 (33.3)	0 (0)	2 (66.7)
CRO	3 (60)	0 (0)	2 (40)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3 (100)
CXM	4 (80)	1 (20)	0 (0)	3 (75)	0 (0)	1 (25)	3 (100)	0 (0)	0 (0)
DXT	5 (100)	0 (0)	0 (0)	1 (25)	0 (0)	3 (75)	1 (33.3)	0 (0)	2 (66.7)
ETP	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	3 (100)
IMP	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)	1 (33.3)	0 (0)	2 (66.7)
LBC	2 (40)	0 (0)	3 (60)	0 (0)	0 (0)	4 (100)	1 (33.3)	0 (0)	2 (66.7)
NA	3 (60)	1 (20)	1 (20)	0 (0)	0 (0)	4 (100)	3 (100)	0 (0)	0 (0)
SXT	1 (20)	3 (60)	1 (20)	1 (25)	0 (0)	3 (75)	2 (66.7)	0 (0)	1 (33.3)
TET	5 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)

Key: ACX = Ampiclox; AUG = Augmentin; CIP = Ciprofloxacin; CRO = Ceftriaxone; CXM = Cefuroxime; DXT = Doxcycline; ETP = Ertapenem; IMP = Imipenem; LBC = Levofloxacin; NA = Nalidixic acid; SXT = Trimethoprim-sulfamethoxazole; TET = Tetracycline.

Table 6. Summary of multiple antibiotic resistance phenotype (MARP) and multiple antibiotics resistance index (MARI) of isolates (n=12).

S. No.	Isolates	MARP	MARI
1		ACX/AUG/CIP/CRO/CXM/DXT/ETP/IMP/LBC/NA/SXT/TET	1.0
2		ACX/AUG/CIP/CRO/CXM/DXT/ETP/IMP/LBC/NA/TET	0.9
3	*E. coli* (n=5)	ACX/AUG/CRO/CXM/DXT/ETP/IMP/NA/SXT/TET	0.7
4		ACX/AUG/CXM/DXT/ETP/IMP/TET	0.5
5		ACX/AUG/DXT/ETP/IMP/TET	0.5
1		ACX/AUG/CIP/CRO/CXM/DXT/SXT/TET	0.6
2	*Salmonella* sp. (n=4)	ACX/AUG/CIP/CRO/CXM/TET	0.5
3		ACX/AUG/CIP/CRO/CXM/TET	0.5
4		AUG/CIP/CRO/TET	0.3
1		ACX/AUG/CIP/CXM/DXT/IMP/LBC/NA/SXT/TET	0.8
2	*Shigella* sp. (n=3)	ACX/AUG/CXM/NA/SXT/TET	0.5
3		ACX/AUG/CXM/NA/TET	0.4

Antibiotic sensitivity testing (AST)

The transmission of antimicrobial-resistant bacteria among the community is a public health threat, leading to hospitalizations, increased treatment costs and/or mortality (Reygaert, 2018). The AST of enteric bacteria from HRW samples is shown in Table 5. *Escherichia coli* isolates were highly resistant to AUG, ACX, DXT, ETP, IMP and TET (100%). This is similar with other studies which showed that *E. coli* isolates exhibited high resistance to combined β-lactam, carbapenem and tetracycline drugs (Breijyeh *et al*., 2020; Ripanda *et al*., 2023). It is reported that ?-lactam drugs have a β-lactam ring, provided with a Zwitterionic structure that protects these drugs from hydrolysis by β-lactamases (Sabrina *et al*., 2021). *E. coli* showed susceptibility to only LBC (60%). The AST of *Salmonella* sp. revealed that all the isolates were highly 100% resistant to AUG, CIP, CRO and TET, but were 100% susceptible to ETP, IMP, LBC and NA. Sabrina *et al*. (2021), reported high resistance of *Salmonella enterica* to TET and CIP. PCR studies on *S. enterica* revealed that tetracycline resistance genes most frequently detected were *tetA* and *tetB* genes, which are often associated with plasmids, transposons, or both, and are often conjugative, highlighting the transference potential of these genes to other bacteria, animals, environments and humans (Frieri *et al*., 2017; Sabrina *et al*., 2021).

Similarly, other research findings reported that *Salmonella* sp. isolated from drinking water exhibited the highest resistance to CRO, AUG and TET (Saeed *et al*., 2019; Okoye *et al*., 2024), and were highly susceptible to NA (Sjolund-Karlsson *et al*., 2014), carbapenem drugs and LBC (Titilawo *et al*., 2024). Hence, carbapenem drugs and LBC can be used as a drug of choice in treating *Salmonella* infections. Antibiotic susceptibility tests of *Shigella* sp. isolates revealed that all the isolates were highly resistant to ACX, AUG, CXM, NA and TET (100%), but were completely susceptible to CRO and ETP (100%), and relatively susceptible to CIP, DXT, IMP and LBC at 66.7%. This agrees with the study of Abaasa *et al*. (2024), who showed *Shigella* sp. were resistant to TET and NA, but were highly susceptible to IMP and ETP. Similarly, the result is consistent with the WHO (2008) recommendation that cases of *Shigella dysenteriae* should be treated with CRO as first-line treatment, and Pivmecillinam, CIP and Azithromycin as second-line treatment. Resistance to AUG (100%) as reported in this study corroborated with the research of Siraj *et al*. (2019), who obtained a resistance rate of 95%. Tetracycline resistance occurs in increasing numbers in pathogenic, opportunistic, and commensal bacteria. *S. dysenteriae* is the first TET-resistant bacterium (Frieri *et al*., 2017). The presence of TET-resistant pathogens limits the use of these agents in the treatment of diseases.

Multiple antibiotic resistance phenotype (MARP) and multiple antibiotics resistance index (MARI)

The MARI is a tool that shows the spread of bacterial resistance in a given population or sample (Afunwa *et al*., 2020). Table 6 shows the MARP and MARI of all isolates, where MAR Index = a/b, where a = number of antibiotics to which a particular organism is resistant to; b = total number of antibiotics tested. MARI ≥ 0.20 implies that the strains of such bacteria originate from an environment where several antibiotics are misused (Manyi-Loh *et al*., 2023). The implication is that relatively large proportion of the bacterial isolates have been exposed to several antibiotics, and thus, have developed resistance to the antibiotics used. All isolates had MAR index greater than 0.20, with *E. coli* having the highest index of 1.0, followed by *Shigella* sp. (0.8) and *Salmonella* sp. (0.6). Malema *et al*. (2018), reported that all the *E. coli* isolates from rooftop rainwater-harvesting tanks in the Eastern Cape, South Africa had MAR index ≥ 0.2. Similarly, Titilawo *et al*. (2024), reported that all pathogenic enteric bacterial isolates from roof-harvested rainwater had MAR index ≥ 0.2, although same set of antibiotics were not used. Hence, high MARI values obtained in this study indicates that there is a great threat for persistent and recurrent infections in the study area.

Conclusion

The results from the physicochemical analysis of HRW samples shows that tested parameters were within the recommended specifications of WHO and NIS. Thus, based on this, HRW can be used for drinking, domestic or agricultural purposes. However, the bacteriological analysis says otherwise, because all the HRW samples exceeded the recommended WHO and NIS limits for total bacterial counts and coliform bacteria, thereby rendering the water samples unfit for human consumption, but could be used for domestic and agricultural purposes. All bacterial isolates were highly resistant to augmentin and tetracycline, but *Salmonella* and *Shigella* species were highly susceptible to ertapenem. Additionally, all the isolates had MAR index ≥0.20, and poses high risk of resistance. It is recommended that 'first flush' diverters be installed within the water collecting system, in order to divert runoff from the rooftops after a period of no rainfall. This usually helps to effectively eliminate bacteriological as well as physical and chemical contaminants. Antibiotics which the isolates were susceptible to, are recommended for the treatment of infections caused by these pathogens. Treatment of HRW and all other forms of water should be a matter of great concern to the people of Oraukwu, and other areas where HRW serve as their main source of drinking water.

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