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PHYSICAL QUALITY AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *Escherichia coli* ISOLATES FROM ROOF-HARVESTED RAINWATER: A MICROCOSM STUDY

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ABSTRACT

Owing to the inadequate supply of potable water in rural settlements in developing countries, roof-harvested rainwater (RHRW) has been favoured as an alternative source of water supply. The present study investigated the physical characteristics and microbial quality of 10 RHRW samples collected from storage tanks in Ikwo community of Ebonyi State, Nigeria. Triplicate water sampling was performed over a 3-month sampling regime. Onsite determination of odour, colour, pH and temperature, and isolation and enumeration of *Escherichia coli* were done using standard procedures. The identity of the isolates was confirmed using molecular techniques, and the susceptibility of the isolates to 11 antibiotics was determined following the Kirby-Bauer disc diffusion assay. Multiple antibiotic resistance phenotypes and indices (MARPs and MARI) were equally determined. Our findings revealed that the RHRWs had unobjectionable odour and were colourless. Across the 3-month sampling, the pH ranged from 6.9 to 7.8 while the temperature was between 24 °C and 29 °C. While *E. coli* was absent in samples E (September), H (August and October) and J (August, September, and October), the highest *E. coli* count in August, September, and October was 37, 32, and 38 cfu/100 mL, respectively. The antibiotic susceptibility test on 40 *E. coli* isolates showed high resistance to ampicillin (100%), aztreonam (98%), cefotetan (97%), nitrofurantoin (90%), imipenem (83%), streptomycin (72%), and ertapenem (69%). Interestingly, the highest MARP, 11 (AK/S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F/TE) (sample B), and the least, 4 (IMI/AMP/CTT/ATM) were recorded in samples B and D, respectively in September. MARI was from 0.42 to 1.00. The physical parameters conform with WHO standards, on the other hand, detection of *E. coli* poses risks of water-borne illness(es) and transmission of antimicrobial resistance. Hence stringent measures including proper treatment, sanitation and hygiene are advocated to safeguard the health of dwellers who depend on this water source for daily life activities.

Keywords: Roof-harvested rainwater, *Escherichia coli*, Antibiotics, Multidrug resistance, Public health.

INTRODUCTION

Roof-harvested rainwater (RHRW) is a principal source of water for households lacking access to potable water in developing nations around the world (Abdulla and Al-Shareef, 2009). Collected directly from rooftops into tanks, it is considered a suitable alternative water source for drinking and various domestic uses (Chukwuma and Ogbu, 2013). Unlike municipal water, RHRW is rarely treated before ingestion because of its presumed quality and safety (Lee *et al*., 2012). However, they may be acidic and/or contaminated by dirt, organic micro-pollutants, metals, pesticides, etc. (Meera and Ahammed, 2006). Additionally, a wide array of pathogens from the faeces of birds, insects, mammals, and reptiles accessing the rooftop and other organic debris are washed following rain events into tanks via roof runoff (Chubaka *et al*., 2018). Hence, drinking untreated RHRW poses a significant public health risk to consumers (Ahmed *et al*., 2008; Simmons *et al*., 2001).

Unsafe waters are responsible for many disease burdens, including morbidity and mortality (Ali *et al*., 2009), and increase the risk of diarrhoea, chronic undernutrition because of severe diarrhoea, and childhood stunted growth (Wolf *et al*., 2023). Thus, the quality of water intended for drinking and other human usage must meet the standard of the World Health Organisation (Titilawo *et al*., 2023). The choice of the physicochemical parameters to be tested depends solely on the purpose of water use (Patil *et al*., 2012). For microbial quality assessment, *Escherichia coli*, a common enteric bacterium and

the indicator organism of faecal pollution must be absent (USGS, 2018). *E. coli* is mostly important in transmitting waterborne diseases and causes high mortality in children less than 5 years old (Chissaque *et al*., 2018).

Antimicrobials are significant in the therapy of bacterial infections (Kolář, 2022). However, the increasing development of resistance to available drugs poses a major challenge in healthcare settings (WHO, 2023). The unwarranted and indiscriminate administration of antibiotics in infection prevention and treatment, growth boosting in veterinary and human medicine are the foremost drivers of the spread and transmittability of resistance traits among pathogenic and resident floral bacteria (Ayukekbong *et al*., 2017). Furthermore, complex social classes and chains of behaviour observable in people encourage the spread of drug-resistant microorganisms (Medina and Pieper, 2016). Water milieus are also significant in the spreading of microorganisms among people, faunas and the environment (Amaya *et al*., 2012).

In Ikwo community, Ebonyi State, Nigeria, the non-availability, and accessibility of clean, potable water to the inhabitant occasioned the dependency on RHRW, especially during the rainy seasons. Unlike Esan West Local Government Area, Edo State (Ezemonye *et al*., 2016), Ikole Local Government Area, Ekiti State (Adeyeye *et al*., 2020), and some parts of Anambra State, Southeast (Chukwuma *et al*., 2014) Nigeria, the quality of RHRW used for drinking and other purposes in Ikwo settlement is unknown. This study was aimed at investigating the physical parameters, and antibiogram of *E. coli* obtained from RHRW in Ikwo community, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Description of Study Areas

The investigation was conducted at Ikwo community, located at Longitude 6.03'11.38"N and Latitude 80.09' 46.22"E, Ikwo Local Government Area (LGA), Ebonyi State, Southeastern Nigeria. Ikwo community covers an area of 500 km and shares boundaries with Abakaliki and Ezza LGAs. The community has a subtropical climate with an abundance of rainfall. In the rainy season, luxurious flora, dense woodlands, pools, and minor ponds are obvious in the area. Most dwellers are farmers who cultivate crops such as yams, and cassava. They are also considered the major producers of Abakaliki rice and palm wine.

Collection of Water Samples

RHRW samples were collected from storage tanks in 10 homes in the Ikwo community, Ebonyi State, Southeast Nigeria. Outlet taps were wiped with ethanol and allowed to run for 30 to 60 s before collecting water samples into sterile 500 mL plastic containers. Triplicate sampling was done between August and October 2021. Samples were placed on ice packs, transported, and analyzed within 24 h.

Physical Parameters of the Water Samples

Temperature and pH were measured in situ with a thermometer and standardized pH meter. Colour and odour examinations were carried out using the sense organs as suitable. Precisely, 100 mL of each water sample was poured into a sterile beaker, mixed thoroughly for froth appearance, and thereafter left to settle. The beaker was observed under bright light to detect the presence of particulate matter and brought close to the nose for a likely offensive odour.

Isolation and Phenotypic Identification *of E. coli*

This was done according to APHA (2012). One hundred millilitres (100 mL) of each water sample was filtered through a sterile membrane filter unit, and the membrane filter was placed aseptically on sterile eosin methylene blue (EMB) agar plates. The Petri dishes were incubated at 37 ºC for 24 h and observed for colonies with a characteristic metallic green colour. To obtain pure cultures, distinct colonies were subcultured on freshly prepared nutrient agar (NA) plates. These cultures were then stored on NA slants for further study.

Molecular confirmation of *E. coli* isolates

DNA extraction

The boiling method was employed for DNA extraction (Titilawo *et al*., 2015). About 2-3 colonies of *E. coli* were picked from an 18-24 h pure culture and dispensed in Eppendorf tubes containing 200 µL of sterile distilled water. The

suspension was boiled for 10 min in a water bath, and centrifuge for 10 min at 1200 rpm. Five microlitres (5 µL) of the supernatant containing the DNA was stored at -10°C for further analysis.

DNA amplification

A pair of *uid*A gene primers F –(AAAACGGCAAGAGAAAAAGCAG) and R (ACGCGTGGTTAACAGTCTTGCG) (Titilawo *et al.*, 2023) specific for the identification of *E. coli* was used for polymerase chain reaction. The PCR reaction was carried out in a final volume of 25 µL containing DNA template (5 µL), master mix (12.5 µL), forward primer (0.5 µL), reverse primer (0.5µL) and nuclease-free water (6.5 µL). The reactions were properly mixed using a vortex, and amplification was performed in a thermocycler (Applied Biosystems, ThermoFisher Scientific, USA) set at initial denaturation temperature of 94 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 8 min.

Gel electrophoresis

Exactly 5 µL of the PCR amplified product were separated by electrophoresis on a 1.0 % agarose gel in 1X TAE buffer. The gel was stained with 10 µL ethidium bromide, analyzed using a GelDoc XR (Bio-Rad, Hercules, CA, USA) transilluminator and photographed with a digital camera.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was carried out following Kirby-Bauer *et al.* (1966). The isolates were screened to a panel of 11 antibiotics (Oxoid, UK) belonging to 8 classes, i.e. aminoglycosides: amikacin (30 µg), streptomycin (100 µg), carbapem: ertapenem (10 µg), imipenem (10 µg), quinolones: ofloxacin (5 µg), norfloxacin (10 µg), penicillin: ampicillin (10 µg), cephalosporin: cefotetan (30 µg), monobactam: azetronam (30 µg), cephalosporin: nitrofurantoin (300 µg), and teracycline: tetracycline (30 µg). Culture suspension was made from an 18-24 h pure culture and standardized to 0.5 McFarland. Precisely, 100 µL of the suspension was inoculated onto a solidified Muller Hinton agar plate by spreading. The antibiotic discs were placed on the agar surface and incubated for 18 h. The zones of inhibition were read in millimetres and interpreted as susceptible, intermediate or resistant according to CLSI (2021). The incidence of antibiotic-resistant isolates was evaluated using the equation: (a/b)*100%. Where 'a' is the sum of isolates resistant to a drug and 'b' is the sum of isolates from the sample.

Multiple antibiotics resistance phenotypes (MARPs)

The MARPs were determined for isolates that exhibited resistance to three or more drugs as previously described (Titilawo *et al.*, 2015).

Determination of Antibiotic Resistance Indices (ARI)

The antibiotic resistance index (ARI) for each sample was assessed using the formula:

ARI = X /Y (Z)

Where, 'X' is the sum of resistant *E. coli* recorded, 'Y' is the quantity of *E. coli*, and 'Z' is the sum of antibiotics employed in the experiment (Titilawo *et al.*, 2015).

Multiple Antibiotic Resistance Indices (MARI)

This is the proportion of antibiotics to which an isolate was resistant to the sum of drugs the *E. coli* was exposed to i.e.

MARI = m/n

Where 'm' is the number of drugs an isolate is resistant to, and 'n' is the sum of the antibiotics used (Titilawo *et al.*, 2015).

RESULTS AND DISCUSSION

In the determination of water quality, physical and microbiological characteristics are essential (Titilawo *et al.*, 2020). The 10 RHRW collected over a 3-month sampling period were colourless and had an unobjectionable odour (Table 1). Our findings agree with Adeyeye *et al.* (2020) who reported an unobjectionable odour and colourless RHRW in Ikole Local Government Area, Ekiti State, Nigeria. In August, September, and October, the pH of the waters ranged from 6.9 to 7.8, 6.8 to 7.6, and 7.0 to 7.8, respectively while the temperature was between 24 °C and 28 ºC in August and September, and 25 °C and 29 °C in October (Table 1). The data obtained are in line with the WHO recommended limit for drinking water, i.e. pH: 6.7 to 8, temperature: 25°C to 30 °C (WHO, 2011) and previous researchers (Frichot *et al.*, 2021; Grabowski *et al.*, 2023).

Table 1: Physical parameters of the RHRW across the 3-month sampling

Sample code	August, 2021				September, 2021				October, 2021			
	Odour	Colour	pH	Temp. (°C)	Odour	Colour	pH	Temp. (°C)	Odour	Colour	pH	Temp. (°C)
A	Odourless	Colourless	7.3	28	Odourless	Colourless	7.4	28	Odourless	Colourless	7.3	29
B	Odourless	Colourless	6.9	26	Odourless	Colourless	6.8	27	Odourless	Colourless	7.0	28
C	Odourless	Colourless	7.1	24	Odourless	Colourless	7.1	25	Odourless	Colourless	7.0	26
D	Odourless	Colourless	7.4	27	Odourless	Colourless	7.4	28	Odourless	Colourless	7.2	28
E	Odourless	Colourless	7.1	28	Odourless	Colourless	7.2	27	Odourless	Colourless	7.3	27
F	Odourless	Colourless	7.5	26	Odourless	Colourless	7.5	25	Odourless	Colourless	7.2	26
G	Odourless	Colourless	7.6	25	Odourless	Colourless	7.5	24	Odourless	Colourless	7.3	25
H	Odourless	Colourless	7.3	28	Odourless	Colourless	7.2	27	Odourless	Colourless	7.1	26
I	Odourless	Colourless	7.8	25	Odourless	Colourless	7.6	25	Odourless	Colourless	7.8	26
J	Odourless	Colourless	7.3	28	Odourless	Colourless	7.4	28	Odourless	Colourless	7.3	28

Escherichia coli detected in the RHRW varied in the 10 samples across the three months of sampling. The highest *E. coli* count in August (37 cfu/100 mL), September (32 cfu/100 mL), and October (38 cfu/100 mL) was observed in samples A, A and D, and G, respectively. On the other hand, no *E. coli* was detected in samples E (September), H (August and October), and J (August, September, and October) (Figure 1). Our finding corroborates Chukwuma *et al.* (2014), who reported varying levels of microbial contamination in RHRW from Oko, Orumba North, Anambra State. The detection of *E. coli* in some of the samples signifies faecal contamination making them unfit for human consumption (Adeyemi *et al.*, 2022). According to Grabowski *et al.* (2023), RHRW possesses good physical parameters, but often contains high levels of microbial contaminants. Microbial contamination of RHRW is generally associated with the faecal dropping of animals, mostly birds, on rooftops (Evans *et al.*, 2006).

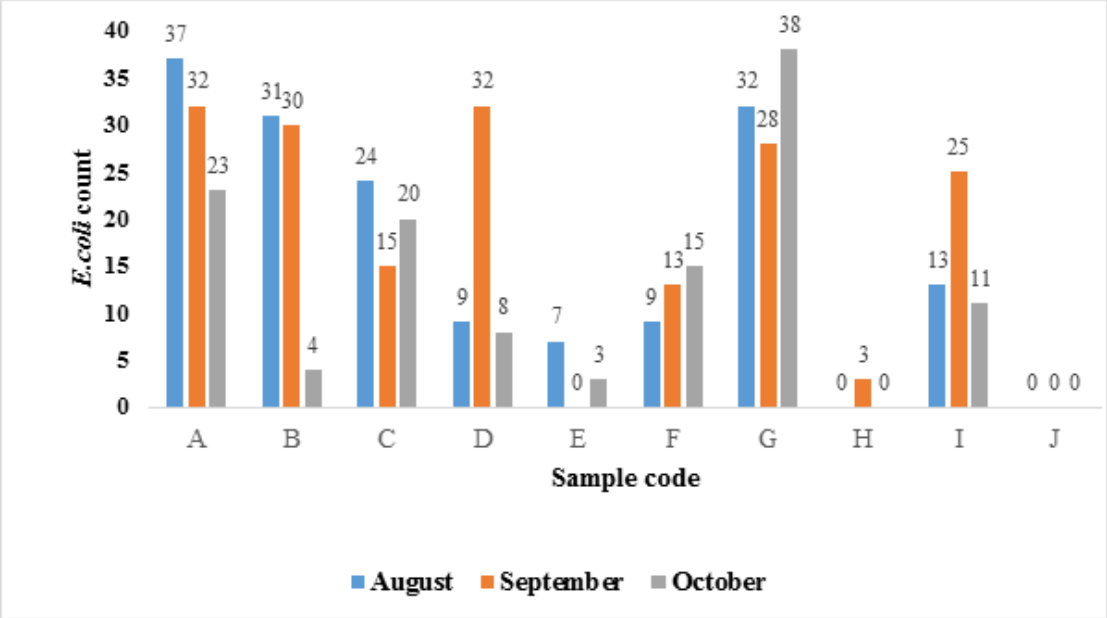

Figure 1: Frequency of *E. coli* in the RHRW across the three months of sampling.

Exactly 40 *E. coli* isolates were selected for further study, and their identity was confirmed using a specific primer, *uid*A. The gel electrophoresis showed a band size of 147 bp for all the isolates (Figure 2). Our observation is in line with earlier works (Titilawo *et al.*, 2015; Titilawo *et al.*, 2023).

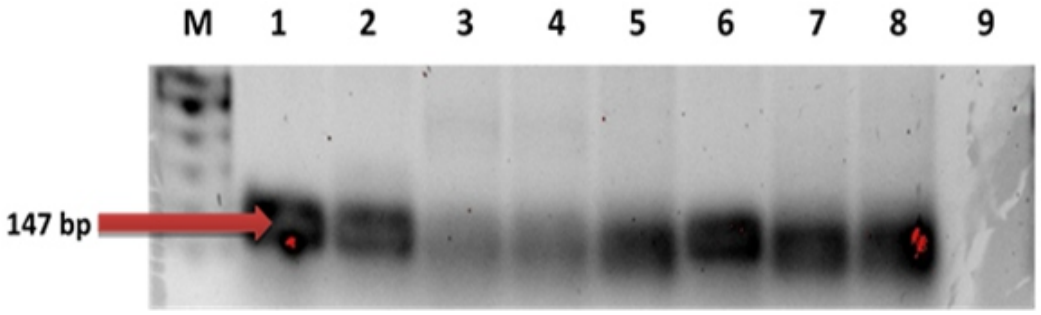

Figure 2: Gel image of *E. coli* isolates from RHRW after amplification of *uid*A gene (147bp). Lanes 1: positive control, Lane 2 to 8: representative *E. coli* isolates; Lane 9: negative control; Lane M: molecular weight maker (100 bp).

The resistance of the isolates to the 11 antibiotics ranged from 7% to 100%. The recovered *E. coli* showed high resistance to ampicillin (100%), aztreonam (98%), cefotetan (97%), nitrofurantoin (90%), imipenem (83%), streptomycin (72%), and ertapenem (69%). Others were in descending order: amikacin (47%) > tetracycline (44%) > norfloxacin (22%), and ofloxacin (7%) (Figure 3). Our observation disagrees with Chidamba and Korsten (2015), who reported low resistance to ampicillin (22.79%) as against 100% in this study. Recently, Alanazi et al. (2018) and Titilawo *et al.* (2023) reported an increasing resistance rate of *E. coli* to ampicillin, despite being a drug of choice. Illicit administration of ampicillin in Ikwo community may be responsible for the observation in this work. Other work noted resistance to cephalothin (76%), tetracycline (51%), ampicillin (50%), and streptomycin (40%) (Malema *et al.*, 2018). The highest susceptibility was observed among *E. coli* isolates to ofloxacin (81%). This suggests the limited exposure arising from low administration of the drug. Others recorded less than 40% susceptibility, including amikacin (32%), norfloxacin (33%), tetracycline (21%), streptomycin and imipenem (17%) (Figure 3).

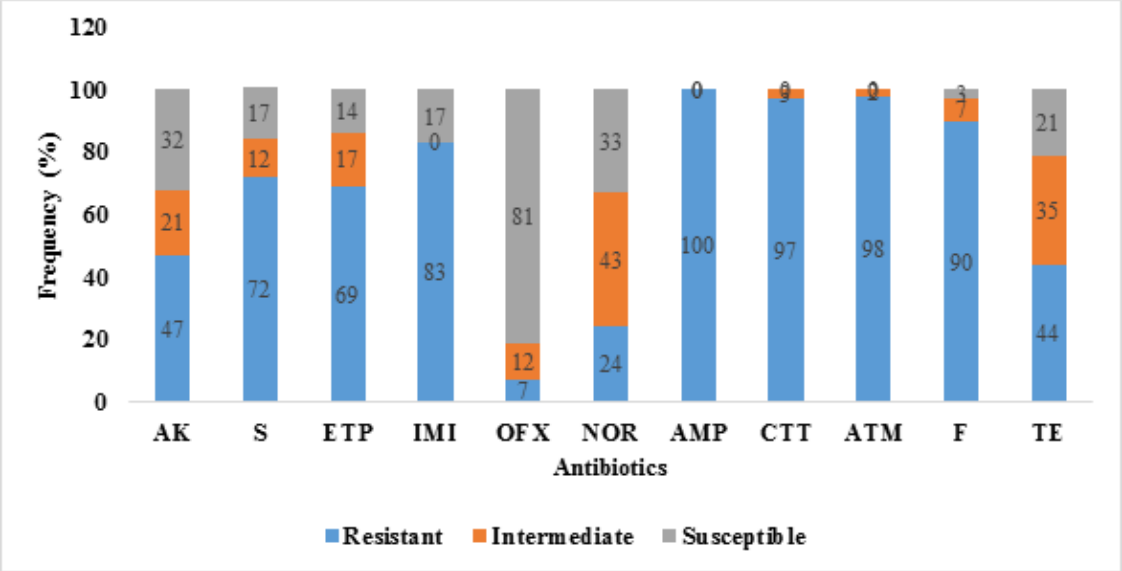

Figure 3: Antibiogram of *E. coli* isolates from RHRW. AK: Amikacin, S: Streptomcin; ETP: Ertapenem, IMI: Imipenem, OFX: Ofloxacin, AMP: Ampicillin, NOR: Norfloxacin, CTT: Cefotetan, ATM: Azetronam, F: Nitrofurantoin, TE; Tetracycline, R: Resistant: I: Intermediate, S: Susceptible

The MARPs generated for the *E. coli* isolates are shown in Table 2. All the isolates investigated (100%) were multidrug-resistant, ranging from 4 (IMI/AMP/CTT/ATM) or 12 drugs (Table 2). This is in variance with Malema *et al.* (2018), who reported 52% multi-resistance *E. coli* from RHRW with MARPs between 3 and 9. Our result signals misuse or unnecessary usage of antibiotics for therapy of medical and veterinary bacterial infections (Ramesh *et al.*, 2010; Titilawo *et al.*, 2015). In August, the lowest and highest MARP were 6 (ETP/IMI/NOR/AMP/ATM/F) and 10 (S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F/TE) in sample E. For the month of September, the lowest MARP 4 (IMI/AMP/CTT/ATM) was in sample D and the highest, 11 (AK/S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F/TE) in sample B. Interestingly, in October, the lowest MARP was 7, and it was observed in locations A, D, F, G, and I. The highest MARP, 9 (AK/S/ETP/IMI/AMP/CTT/ATM/F/TE) was however observed in sample G (Table 2). Previous work also reported varying MARPs in *E. coli* isolated from different RHRW samples in South Africa (Malema *et al.*, 2018). This infers the ineffectiveness of the drugs when administered in the treatment of infections arising from the consumption of the RHRW. ARI ranged from 0.010 to 0.025, while MARI was from 0.42 to 1.00 (Table 2). Our outcome (MARI) is in line with Chidamba and Korsten (2015) and Malema *et al.* (2018), who also noted a MARI greater than 0.2, suggesting that the isolates are from a high-risk source where antibiotics are overly used. This is an indication of severe health risk to the Ikwo community of Ebonyi State, as many households rely on RHRW to meet their daily water needs.

Table 2: MARPs, ARI and MARI of the *E. coli* Isolates.

Sampling location	MARP of *E. coli* isolates	NRA	ARI	MARI
	August (*n*=12)			
B	AK/S/ETP/IMI/AMP/CTT/ATM/F	8	0.018	0.75
	AK/S/ETP/IMI/NOR/AMP/CTT/ATM	8	0.018	0.75
C	S/ETP/IMI/NOR/AMP/CTT/ATM/F	8	0.018	0.75
	AK/S/ETP/IMI/AMP/CTT/ATM	7	0.016	0.67
E	S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F/TE	10	0.022	0.92
	ETP/IMI/NOR/AMP/ATM/F	6	0.014	0.58
F	AK/ETP/IMI/AMP/CTT/ATM/F	7	0.016	0.67
	AK/ETP/IMI/AMP/CTT/ATM/F	7	0.016	0.67
G	AK/S/ETP/IMI/AMP/CTT/ATM/F/TE	9	0.020	0.83
	S/ETP/IMI/AMP/CTT/ATM/F/TE	8	0.018	0.75
I	AK/S/ETP/IMI/AMP/CTT/ATM/F/TE	9	0.020	0.83
	ETP/IMI/AMP/CTT/ATM/F/TE	7	0.016	0.67
	September (*n*=16)			
A	AK/ETP/IMI/NOR/AMP/CTT/ATM/F	8	0.018	0.75
	ETP/IMI/AMP/CTT/ATM/F	6	0.014	0.58
B	AK/S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F/TE	11	0.025	1.00
	S/ETP/IMI/AMP/CTT/ATM/F/TE	8	0.018	0.75
C	S/IMI/AMP/CTT/ATM/F/TE	7	0.016	0.67
	AK/IMI/AMP/CTT/ATM/F/TE	7	0.016	0.66
D	AK/S/IMI/AMP/CTT/ATM/F	7	0.016	0.67
	IMI/AMP/CTT/ATM	4	0.010	0.42
F	AK/S/IMI/NOR/AMP/CTT/ATM/F/TE	9	0.020	0.83
	S/IMI/AMP/CTT/ATM/F/TE	8	0.016	0.67
G	AK/S/ETP/IMI/OFX/NOR/AMP/CTT/ATM/F	10	0.022	0.92
	AK/S/ETP/IMI/AMP/CTT/ATM/F/TE	9	0.020	0.83
H	S/IMI/AMP/CTT/F	5	0.0125	0.50
	AK/IMI/AMP/CTT/ATM/F	6	0.014	0.58
I	EPT/IMI/NOR/AMP/CTT/ATM/F	7	0.016	0.67
	S/ETP/IMI/AMP/CTT/ATM/F/TE	8	0.018	0.75
	October (*n*=12)			
A	AK/ETP/IMI/AMP/CTT/ATM/F	7	0.016	0.67
	AK/S/ETP/IMI/AMP/CTT/ATM/F	8	0.016	0.67
B	AK/ETP/IMI/AMP/CTT/ATM/F/TE	8	0.018	0.75
	S/ETP/IMI/AMP/CTT/ATM/F/FE	8	0.018	0.75
D	AK/S/IMI/AMP/CTT/ATM/TE	7	0.016	0.67
	S/ETP/IMI/AMP/CTT/ATM/F	7	0.016	0.67
F	AK/S/IMI/AMP/CTT/AMT/F	7	0.016	0.67
	AK/S/IMI/AMP/CTT/ATM/F/TE	8	0.018	0.75
G	AK/S/IMI/AMP/CTT/ATM/F	7	0.016	0.67
	AK/S/ETP/IMI/AMP/CTT/ATM/F/TE	9	0.020	0.83
I	S/ETP/NOR/AMP/CTT/ATM/F	7	0.016	0.67
	AK/S/IMI/AMP/CTT/ATM/TE	7	0.016	0.67

AK: Amikacin, S: Streptomycin; ETP: Ertapenem, IMI: Imipenem, OFX: Ofloxacin, AMP: Ampicillin, NOR: Norfloxacin, CTT: Cefotetan, ATM: Azetronam, F: Nitrofurantoin, TE; Tetracycline, NRA: Number of antibiotics to which isolate was resistant, MARP**:** Multiple antibiotic resistance phenotypes, ARI: Antibiotic resistance index, MARI: Multiple antibiotic resistance index

CONCLUSION

The RHRWs investigated in this study contained varying concentrations of *E. coli*, even though the physical characteristics were according to the WHO standards. Our results establish that RHRW are possible reservoirs for antibiotic-resistant *E. coli* strains and consumption of the water poses a major health hazard. The high resistance observed in our isolates to the test antibiotics could lead to difficult-to-treat infections with attendant disease burdens because the isolates are multidrug-resistant. However, ofloxacin may be the preferred drug for treating infections arising from drinking contaminated water. Our findings suggest that the RHRW is not fit for human consumption without adequate treatment. Thus, there is a need for implementing workable strategies, including regular cleaning of the rooftops, avoiding collection of first-flush to minimise faecal contamination, periodic washing of storage, and the adoption of simple disinfection methods to avert contamination with *E. coli* and antibiotic-resistant bacteria. Awareness creation, in addition to the implementation of laws and policies that check illegitimate prescription and administration of antimicrobials will help combat the menace of drug resistance.

CONFLICT OF INTEREST

The authors declare no conflicting interest.

REFERENCES

Abdulla, F. A. and Al-Shareef, A. W. (2009). Roof rainwater harvesting systems for household water supply in Jordan. *Desalination*, 243(1-3), 195-207.

Adeyemi, F.M., Wahab, A.A., Oyelami, C.A., Oyedara, O.O., Titilawo, M.A., Adebunmi, A.A. and Awoniyi, I.O. (2022). Hydrology survey and water quality assessment of water sources in three selected towns in Osun State, Southwest Nigeria. *International Journal of Energy and Water Resources*, 7: 271-284.

Ahmed, W., Huygens, F., Goonetilleke A. and Gardner. T. (2008). Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Applied Environmental Microbiology*, 74, 5490 - 5496.

Ali, E.N., Muyibi, S.A., Salleh, H.M., Salleh M.R.M. and Alam, M. (2009). *Moringa oleifera* seeds as natural coagulant for water treatment. Proceedings of the 13th International Conference on Egyptian Water Technology 2009, March 12-15, 2009, International Islamic University Malaysia, Hurghudah, Egypt, pp: 163-168.

Amaya, E., Reyes, D., Paniagua, M., Calderón, S., Rashid, M-U., Colque, P., Kühn, I., Möllby, R., Weintraub, A. and Nord, C.E. (2012). Antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in Leon. *Nicaragua Clinical Microbiology Infections*, 18(9):E347-E354.

APHA. (2012). Standard Methods for the Examination of Water and Wastewater (22nd ed., p.1496). American Public Health Association, AWWA (American Water Works Association), Water Environment Federation. ISBN-13: 978-087553013

Ayukekbong, J.A., Ntemgwa, M. and Atabe, A.N. (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance and Infection Control*, 6: 47.

Chidamba, L. and Korsten, L. (2015). Antibiotic resistance in *Escherichia coli* isolates from roof-harvested rainwater tanks and urban pigeon faeces as the likely source of contamination. *Environmental Monitoring and Assessment*, 187(7): 405.

Chissaque, A., de Deus, N., Vubil, D. and Mandomando, I. (2018). The epidemiology of diarrhea in children under 5 years of age in Mozambique. *Current Tropical Medicine Reports*, 5:115-124.

Chubaka, C. E., Whiley, H., Edwards, J. W. and Ross, K. E. (2018). Microbiological Values of Rainwater Harvested in Adelaide. *Pathogens*, 7(1): 21.

Chukwuma, E.C. and Ogbu, K.N. (2013). Quality assessment of direct harvested rainwater in parts of Anambra State, Nigeria. *International Journal of Agriculture and Bioscience*, 2(3): 120-123.

Chukwuma, J. N., Nnodu, V. C., Okoye, A. C. and Chukwuma, E.C. (2014). Assessment of roof harvested rainwater in parts of Anambra State for environmental pollution monitoring. *British Biotechnology Journal*, 4(10): 1105-1114.

CLSI (2021). Performance standards for Antimicrobial Susceptibility Testing. CLSI supplement M100, 29th Edition, Wayne, PA, USA.

Evans, C., Coombes, P. and Dunstan, R. (2006). Wind, rain and bacteria: The effect of weather on the microbial composition of roof-harvested rainwater. Water Research 40: 37-44.

Ezemonye, M.N., Isueken, C., and Emeribe, C.N. (2016). Physicochemical and bacteriological characteristics of rainwater harvested from rooftops in Esan-West Local Government Area of Edo State, Nigeria. Journal of Applied Sciences and Environmental Management 20(3):748.

Frichot, J.J.H., Rubiyatno, Talukdar, G. (2021). Water quality assessment of roof-collected rainwater in Miri, Malaysia. *Tropical Aquatic and Soil Pollution* 1(2): 87-97.

Grabowski, T., Jó ´zwiakowski, K., Bochniak, A., Stachyra, P. and Radli ´nski, B. (2023). Assessment of rainwater quality regarding its use in the Roztocze National Park (Poland) -Case Study. *Applied Science* 13: 6110.

Kirby-Bauer, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by single disc method. *American Journal of Clinical Pathology*, 45:4.

Kolář, M. (2022). Bacterial infections, antimicrobial resistance and antibiotic therapy. *Life* (Basel), 12(4): 468.

Lee, J.Y., Bak, G. and Han, M. (2012). Quality of roof-harvested rainwater-Comparison of different roofing materials. *Environmental Pollution*, 162: 422-429.

Malema, M. S., Abia, A., Tandlich, R., Zuma, B., Mwenge Kahinda, J. M. and Ubomba-Jaswa, E. (2018). Antibiotic-resistant pathogenic *Escherichia coli* isolated from rooftop rainwater-harvesting tanks in the Eastern Cape, South Africa. *International Journal of Environmental Research and Public Health*, 15(5): 892.

Medina, E. and Pieper, D.H. (2016). Tackling threats and future problems of multidrug-resistant bacteria. *Current Topics in Microbiology and Immunology*, 398:3-33.

Meera, V. and Ahammed, M.M. (2006). Water quality of rooftop rainwater harvesting systems: A review. *Journal of Water Supply Research and Technology AQUA*, 55: 257-268.

Patil, P.N., Sawant D.V. and Deshmukh, R.N. (2012). Physico-chemical parameters for testing of water: A review. *International Journal of Environmental Science*, 3: 1194-1207.

Ramesh, S., Manivasagan, P., Ashokkumar, S., Rajaram, G. and Mayavu, P. (2010). Plasmid profiling and multiple antibiotic resistances of heterotrophic bacteria isolated from Muthupettai Mangrove Environment, Southeast Coast of India. *Current Research in Bacteriology*, 3:227-237.

Simmons, G., Hope, V., Lewis, G., Whitmore, J. and Gao, W. (2001). Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Research,* 35(6): 1518 – 1524.

Titilawo, M.A., Fatoki, C.O., Titilawo, Y. and Olaitan, J.O. (2023). Assessment of multidrug-resistant phenotypes and detection of ampicillin-resistant determinants among *Escherichia coli* isolates of groundwater origin: case study – Osogbo, Southwest Nigeria. *Sustainable Water Resources Management*, 9: 8.

Titilawo, Y., Sibanda, T., Obi, L. and Okoh, A. (2015). Multiple antibiotics resistance indexing of *Escherichia coli* to identify high risk sources of faecal contamination of water. *Environmental Science and Pollution Research,* 22(14): 10969-10989.

Titilawo, Y., Nwakpa, F., Bankole, S., Nworie, O., Okoro, C., Titilawo M. and Olaitan, J. (2020). Quality audit of drinking water sources in Ikwo rural setting of Ebonyi State, Southeastern Nigeria. *International Journal of Energy and Water Resource*,4 (3): 321-334.

USGS (2018). Bacteria and *E. coli* in water. https://www.usgs.gov/special-topic/water-science-school/science/bacteria-and-e-coli-water?qt-science _center_objects=0#qtscience_center_objects. Assessed August, 2021.

WHO (2023). Antimicrobial resistance.

Wolf, J., Johnston, R.B., Ambelu, A., Arnold, B.F., Bain, R., Brauer, M., Brown, J., Caruso, B.A., Clasen. T., Colford, J.M. Jr., Mills, J.E., Evans, B., Freeman, M.C., Gordon, B., Kang, G., Lanata, CF., Medlicott, K.O., Prüss-Ustün, A., Troeger, C., Boisson, S. and Cumming, O. (2023). Burden of disease attributable to unsafe drinking water, sanitation, and hygiene in domestic settings: a global analysis for selected adverse health outcomes. *Lancet*, 401(10393):2060-2071.