



Assessment of the Drinking Quality of Roof-Harvested Rainwater in Nsukka Metropolis, Nigeria

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Keywords: Roof Harvested Rainwater (RHRW); Drinking water; Antibiotic resistance; *Escherichia coli*; Bacteriological analysis.

Abstract

Roof Harvested Rainwater (RHRW) is a common technique used to collect and store rainwater that runs off from roofs for human use, both in urban and rural environment. The present study aimed at evaluating the antimicrobial susceptibility of *Escherichia coli* isolated from roof harvested rainwater in Odenigwe Edem, Ibagwa Behind Flat, Odim and Green house in Nsukka area of Enugu State, Nigeria. A total of 20 roof harvested rainwater samples were collected from storage media (buckets and tanks) from private and public places in the locations, The bacteriological analysis of the samples was carried out through spread plate and pour plate and the antibiotic resistance of the isolates was determined using Kirby-Bauer disk diffusion assay. The results of storage tanks showed significantly differences in pH and temperature. From the result, 100% of the roof harvested rainwater samples were positive for *E. coli*. Ofloxacin, Peflacin and Ciprofloxacin were antibiotics found to be the most effective against *E. coli*. This study shows that roof harvested rainwater (RHRW) may not be suitable for drinking without treatment but could be used for other domestic purposes, there is also a need for further study on other possible microbial contaminants of RHRW.



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Introduction

Water is vital for life and the well-being of human. However, access to adequate and safe water is a challenge for many people around the world, especially in developing countries. Research has it that about 2.2 billion people lack safely managed drinking water services, and 4.2 billion people lack safely managed sanitation services [13]. These condition poses serious health risks, as waterborne diseases are among the leading causes of mortality around the globe. This National crisis of portable water has given rise to the search for other means of getting water, some of which include Roof harvested rainwater. Rainwater harvesting has proved useful in filling up the gap for the search of portable water, as it offers a comparatively low cost, it can be used for internal applications such as bathing, laundry, toilet flushing [6,7], but most of these areas which make good use of harvested rainwater lack conventional water supply systems as the water is collected either directly or through catchment areas for storage and eventual use.

Rooftop rainwater harvesting is a type of rainwater harvesting that involves capturing rainwater from the roof catchments and storing it in different types of tanks or reservoirs [11]. This type of rainwater can be used for both potable and non-potable purposes, depending on the quality of the harvested rainwater and the method of treatments applied. Rooftop rainwater harvesting also poses some challenges and risks, as the rainwater quality can be affected by various factors, which include: the type of and condition of the roofing material, the bird and animal droppings, the dust and the debris, the atmospheric pollution, and the maintenance of the catchment system [8]. These factors can introduce various contaminants into the harvested rainwater and these contaminants can cause infectious diseases in both humans and animals [15].

Escherichia coli. (*E. coli*), a gram negative, rod-shaped bacterium that normally lives in the intestines of humans and animals, have been detected as a common pathogen in harvested rainwater [2,12]. Most of the strains of *E. coli* are harmless or beneficial to their hosts, while some strains can cause serious diseases such as diarrhea, urinary tract infection, etc. *E. coli* is one of the most common indicators of fecal contamination in water sources, as this implies the presence of other pathogens that may have originated from human or animal feces [4].

The isolation of *E. coli* from roof harvested rainwater is very important as it help assess the potential health risks associated with using the harvested rainwater for different purposes and help evaluate the effectiveness of different treatment methods for improving the harvested rainwater quality.

Materials and method

Study area

Nsukka is a town and a local government area in Enugu State, Nigeria. It is in the Udi Hills at an elevation of about 430 meters above sea level. Nsukka has a tropical wet and dry climate with an average annual rainfall of about 1,800 mm and an average annual temperature of about 26°C. Nsukka is an agricultural-trade center for the yams, cassava, corn, taro, pigeon peas, palm oil and palm kernels produced by the local Igbo people. Nsukka is also the host to the first indigenous university in Nigeria, the University of Nigeria, Nsukka (UNN).

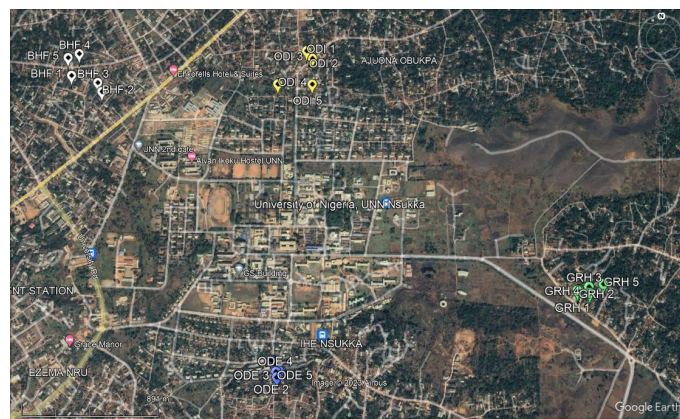


Figure 1: Map of Nsukka Local government, Enugu state, Nigeria.

Source: www. Google.com

Sampling

20 samples were collected randomly from houses located at Odenigwe, Behind Flat, Odim, Ibagwa, Edem and Greenhouse in Nsukka Local Government, Enugu State, into sterile sample bottles aseptically and taken to the University of Nigeria, Nsukka Microbiology General laboratory for analysis. The collection was done in batches over a period of 2 weeks to assess the presence of the bacterial specie (*Escherichia coli*) in the roof harvested rainwater samples in the above areas. A total of 20 samples were collected. They were sub-cultured and subsequently identified using Mueller Hinton Agar (MHA) and Eosin Methylene Blue Agar (EMB); and pure cultures were stored in the refrigerator.

Microbiological assessment

The quantitative evaluation of bacterial contamination was conducted using the pour plate and spread plate method. The sample bottles were sterilized by autoclaving at 121°C for 15 mins, 24 hours before using them to collect samples. The sterile sample bottles were kept in a sterile cooler to avoid contamination. Each sterile sample bottles were gradually filled with the harvested rainwater sample contained in either a metal tank, a plastic tank, bucket or water drums as the case may be and kept back in the cooler. The harvested rainwater samples were collected randomly from different locations within a given area.

Using a pH meter and a thermometer, the physiochemical properties of each of the samples were recorded. To selectively isolate *E. coli* from the samples, the pour plate was used to inoculate liquid samples on Eosin Methylene Blue (EMB) agar media while the spread plate was used to evenly spread cells to ensure growth of the isolated separate colonies. The Petri dishes were labeled with relevant information such as location, date, the media used, and the culture being inoculated. The agar medium is allowed to solidify, and the petri dish is incubated upside down at the appropriate temperature and time. Two-fold serial dilutions were prepared to reduce the microbial colony load to a range of 20-300 CFU/ml.

The clinical specimen where we spread the bacteria gently on the whole culture media surface, was inoculated with the help of a sterile spreader. Newly prepared EMB Agar were distributed in petri-dishes. Distinct colonies were obtained and repeatedly subculture aseptically using the streak plate method. This was done by picking single colonies using a sterile wire loop and streaking on the agar plate. The plates were inoculated at 37°C for 24 hours to enable maximum growth of pure

cultures.

EMB Agar was also prepared in slants in bijou bottles, autoclaved and allowed to cool. The pure isolates gotten from the sub culturing were transferred into the slants aseptically and kept in the refrigerator at 4°C to serve as stock culture for subsequent tests during identification of organism. The plates were later incubated at 35°C for 24 hours. To determine the *E. coli* quantities, we manually counted all the bluish black with a metallic green colony.

Identification of bacterial isolates

Biochemical tests carried out following the method described by Ezeonu et al. (2011). Catalase test, citrate utilization test, indole test, methyl red test, Voges Proskauer and sugar fermentation test using Triple Sugar Iron (TSI) agar: lactose, glucose, sucrose as sugars.

Antimicrobial susceptibility test for bacterial isolates

Sensitivity test was performed using the standard disk diffusion method according to Birks et al. (2004). Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates was used to perform antimicrobial susceptibility of all *Escherichia coli* isolates. The bacterial cell suspension was prepared at 1×10^8 CFU/ml following the McFarland 0.5 turbidity standard. The antibiotic disk used was manufactured by Optun Laboratories Nig. Ltd. The zone of growth inhibition for each organism was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

For *Escherichia coli*: Ofloxacin (10 MCG), Augmentin (30 MCG), Peflacin (10 MCG), Ceftazidime (30 MCG), Gentamycin (10 MCG), Ciprofloxacin (10 MCG), Ceporex (10 MCG), Ceftriaxone (30 MCG), Streptomycin (30 MCG), Cefuroxime (30 MCG). The antimicrobial disk, after being purchased from a reputable supplier, was then properly stored so as to produce reproducible results. The antimicrobial agent diffused from the disk to the medium and the growth of the organism was inhibited at a distance from the disk while resistant strains had smaller or no zones of inhibition. The MAR index (multiple antibiotic resistance) was also determined and calculated.

Results

Physicochemical properties of roof-harvested rainwater

The physicochemical properties were evaluated according to WHO standard where a pH of range 6.5-8.5. as considered as compliant and a Temperature of 25-26.8°C is considered as WHO complaint of drinking according to the WHO 2017 *Guidelines for Drinking-Water Quality (GDWQ)*. This is presented in Table 1 below.

Subculture and characterization of bacterial isolates

Each sample subculture into EMB. media selective produced greenish metallic sheen colonies after incubation, and after the biochemical testing, the colonies tested positive to catalase test, citrate test, indole test, methyl red test and negative to Voges Proskauer test, while the butt of the test tube showed a black precipitate indicating the production of sulphide. These are indicative of the physical and biochemical characteristic of *E. coli* on EMB media as shown in Table 2 below.

Table 1: Physicochemical properties of roof-harvested rainwater.

Sample	pH	Temperature	WHO Compliance
1	6.8±0.03	32±0.01	NO
2	7.1±0.02	29±0.02	No
3	7.2±0.05	26.2±0.03	YES
4	6.9±0.01	25.9±0.11	YES
5	7.0±0.01	29.0±0.03	NO
6	6.1±0.02	32±0.02	No
7	7.4±0.01	31±0.04	NO
8	6.3±0.03	27±0.05	YES
9	7.3±0.02	32±0.02	NO
10	8.7±0.01	32±0.1	NO
11	7.0±0.09	26.1±0.1	YES
12	8.6±0.05	32±0.4	NO
13	6.5±0.07	26.1±0.03	YES
14	7.2±0.09	26±0.02	YES
15	7.3±0.09	25±0.05	YES
16	7.5±0.06	26±0.02	YES
17	6.5±0.13	25±0.03	YES
18	6.0±0.27	33±0.05	NO
19	6.2±0.01	30±0.03	NO
20	7.4±0.21	33±0.04	NO

Table 2: Characterization of bacterial isolates obtained from the samples.

Characterization	Result
Color change	Greenish metallic sheen colonies
Catalase test,	Positive
citrate test,	Positive
indole test,	Positive
methyl red test	Positive
Voges Proskauer test	Negative

Table 3: Antimicrobial susceptibility test of microbial isolates.

Number of resistance pattern	Antibiotics	Number of Isolates	MAR I
3	CEP, AU, CTZ	8	0.3
3	CEP, AU, CTZ	8	0.3
3	CEP, AU, CTZ	8	0.3
3	CEP, AU, CTZ	8	0.3
7	CEP, TRX, S, CEF, AU, CTZ, CN	1	0.7
3	CEP, AU, CTZ	8	0.3
3	CEP, AU, CTZ	8	0.3
3	CEP, AU, CTZ	8	0.3
2	AU, CTZ	1	0.2

Note: S: Susceptibility; I: Intermediate; R: Resistance. $S \geq 20$ mm, $I = 15-19$ mm, $R \leq 14$ mm.

OFX: Ofloxacin (10 MCG), AU: Augmentin (30 MC), PEF: Peflacin (10 MCG), CTZ: Ceftazidime (30 MCG), CN: Gentamycin (10 MCG), CPX: Ciprofloxacin (10 MCG), CEP: Ceporex (10 MCG), TRX: Ceftriaxone (30 MCG), S: Streptomycin (30 MCG), CEF: Cefuroxime (30 MCG).

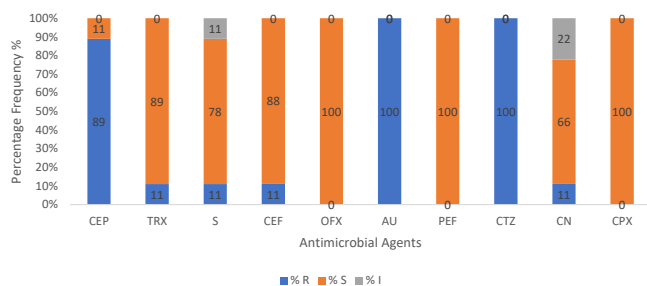


Figure 2: Percentage frequency of antimicrobial susceptibility patterns of *Escherichia coli*.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was carried out using gram negative multi disk containing the following drugs: Ofloxacin (10 MCG), Augmentin (30 MCG), Peflacin (10 MCG), Ceftazidime (30 MCG), Gentamycin (10 MCG), Ciprofloxacin (10 MCG), Ceporex (10 MCG), Ceftriaxone (30 MCG), Streptomycin (30 MCG), Cefuroxime (30 MCG). Table 3 shows the antibiotics susceptibility profiles of the Nine *E. coli* isolates tested with ten different antibiotics. The Clinical Laboratory Standard Institute (CLSI, 2020) was used as an acceptable standard to compare the zone of inhibition.

Discussion

Roof Harvested Rainwater (RHRW) is a common technique used to collect and store rainwater that runs off from roofs for human use, both in urban and rural environment. Rooftop rainwater harvesting poses some challenges and risks, as the rainwater quality can be affected by various factors, which include: the type of and condition of the roofing material, the bird and animal droppings, the dust and the debris, the atmospheric pollution, and the maintenance of the catchment system. These factors can introduce various contaminants into the harvested rainwater and these contaminants can cause infectious diseases in both humans and animals [8,15].

From the results of this study, the resistance displayed by most of the organisms to the various antibiotics which were tested, is worrisome, especially the resistance that was demonstrated to three (3) of the antibiotics CEP, AU and CTZ. The resistance of *Escherichia coli* to some antibiotics used is of great health concern. The variation in susceptibility and resistance of the isolates to the different antibiotics could be attributed to the difference in the concentration of antibiotics and drug resistance transfer [9].

A high resistance of Augmentin and Ceftazidime was observed for *E. coli* isolates. Antimicrobial resistance in *E. coli* has increased worldwide over the years, and its susceptibility patterns show substantial geographic location as well as differences in the population and environment. The overall resistance of *E. coli* to antimicrobials can be said to be high. The result is consistent with the findings of previous studies [1].

Antibiotics play a very important role in decreasing the level of diseases, illnesses or death associated with bacterial infections in humans and animals. Nevertheless, the use of antibiotics as growth promoters and therapeutics purposes have been the major driving force behind the emergence and spread of drug-resistance bacteria among pathogenic and non-pathogenic bacteria strains [3,10]. In this study, Ofloxacin, Peflacin and Ciprofloxacin were found to be the most effective antimicrobials against *Escherichia coli*.

Conclusion

This study has shown that 70% of the roof harvested rainwater (RHRW) from the study area is not physicochemical suitable for drinking according to the WHO drinking water guideline and *E. coli* are present in the water tanks in the study locations. This suggests that foods prepared using this water without proper boiling, are likely to be contaminated by these organisms and may predispose the consumers to food poisoning (gastroenteritis). *Escherichia coli* is known as a commonly used indicator of fecal contamination in water sources and is therefore, widely used as a tool for assessing the risk of water borne diseases. However, it should be noted that *E. coli* is just one of the several possible indicators of water quality, and its presence alone does not necessarily mean that a water source is unsafe. The high concentration of *E. coli* in the water samples studied, proves the potential public health hazards as most of these roofs harvested rainwater (RHRW) does not undergo any treatment before consumption. The water samples are therefore deduced as major reservoirs for potentially pathogenic *E. coli* and other microorganisms.

Author declarations

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Conflicts of interest

All authors declare that they have no conflict of interest associated with this research article.

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