Greener Journal of Environment Management and Public Safety
ISSN: 2354-2276
Vol. 12(1), pp. 16-21, 2024

https://gjournals.org/GJEMPS

# The Role of Rain Water Harvesting for Domestic Use in the Prevalence of *Escherichia coli* (E. coli) Diseases in Otuoke Community

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**ARTICLE INFO**

***Article No.:*** *042124050*
***Type*:** *Research*
***Full Text:*** *PDF, PHP, HTML, EPUB, MP3*

***Accepted:*** *22/04/2024*
***Published:*** *29/04/2024*

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***Keywords:*** *Rain Water Harvesting, Domestic Use, Prevalence of Escherichia coli Diseases, Pathogens in Rainwater.*

**ABSTRACT**

**This study looks at *E. coli* and other pathogenic microorganisms from rain water harvested from roof of houses for domestic use. It determines the overall prevalence of *E. coli* in rainwater harvested from the roof of houses for domestic use and gives recommendation that could help to reduce the health effects of consuming rainwater harvested from roof of houses. Humans', animals, and plants all depend on water; without it, life cannot exist. An adequate supply of clean water is a basic requirement for a community's socioeconomic growth. High-quality drinking water is also essential to everyone's health and welfare. Samples used for this research work were rain water collected from three (3) different locations in Otuoke community namely Azikel Road, Hospital Road, Federal University Otuoke West Campus (FUO). The water samples were collected from rain water reservoirs, and a control gotten directly from the rain. Three (3) of the samples were positive for *E. coli* with Azikel Road sample having the highest microbial load, followed by Hospital Road, and FUO West Campus sample, carrying the lowest. For Salmonella, all of the samples had Negative results. However, other opportunistic pathogens such as *Psuedomonas aeruginosa, Staphylococcus aureus,* and yeast were present in all of the samples, including the Control. *Escherichia coli* poses a health risk to end-user communities that consumes rain water from rooftops, especially when used for drinking, cleaning of the home, garden hosing, washing laundry by hand, or when accidentally consumed by human beings.**

## INTRODUCTION

Humans, animals, and plants all depend on water; without it, life cannot exist. An adequate supply of clean water is a basic requirement for a community's socioeconomic growth. High-quality drinking water is also essential to everyone's health and welfare. Acceptable water quality is defined as the absence of bacteria of fecal origin that can cause human diarrhoea and other life-threatening diseases (like typhoid fever), chemicals (like heavy metals) or other substances that can harm human health, and when the water does not taste or smell unpleasant (Agbede *et al*., 2017).

In majority of developing nations, rainwater harvesting is a common practice. Rainwater harvesting is defined as the process of directly collecting rainfall for domestic uses, irrigation or refilling groundwater storage (Lade and Oloke, 2013). The technique of gathering rainwater and storing it for use at a later time on the surface, below ground, in the soil, or in reservoirs is known as rainwater harvesting. Rainwater harvesting also refers to the practice of using surface runoff from rainfall to be used for irrigation in farms and houses (ABS 2010, ADWG 2004).

In many parts of the world, collected rainwater is a significant source of water for domestic use. Rooftop water is collected and stored in concrete surface tanks, concrete subsurface tanks, or other types of water storage containers. Rooftop rainwater collection has the potential to create water of exceptional quality if the rooftop is clean, impermeable, and made of non-toxic materials (Lee *et al*., 2010). There is a growing interest in rainwater harvesting as a substitute for surface and ground water due to the rising scarcity of potable water in various parts of the world. As a substitute source of potable and non-potable water resources, rainwater harvesting has gained more attention globally (Hatibu *et al*., 2006; Ghisi and Ferreira, 2007). In Nigeria, rainwater harvesting is practiced by about 80% households (Lade and Oloke, 2013).

This strategy reduces water demand while encouraging energy and water conservation. It also stimulates climate change adaption measures. Rainwater is the main water supply for residents in some rural areas in Nigeria, who utilize it for drinking and other domestic needs (Grandet *et al*., 2010). Even though using rainwater has some prospective advantages over other sources, it is generally disregarded as a source of drinkable water due to worries about the water quality (Dobrowsky *et al.,* 2014). Heavy metals, nutrients, and pathogens can all be present in substantial amounts in rainwater (Khayan *et al.,* 2019; Hassan, 2023; Bełcik *et al.,* 2024).

Dust, insect and bird feces, and even small animals present on rooftops hold a variety of germs, including human pathogens (Ahmeda *et al.,* 2012; Ahmed *et al*., 2014). During rainfall, pathogen-carrying material such as feces and other objects can be washed into rainwater harvesting tanks, reducing the quality of the water (Pachepsky *et al*., 2011). The presence of bacteria, viruses, and parasites, many of which potentially cause waterborne diseases, has been associated with rainwater harvesting (Ahmed *et al.,* 2012b; Ahmed *et al*., 2014). Kanzler et al., 2008; Hageskal *et al*., 2009) had that the existence of organisms in rainwater itself, atmospheric deposition of organisms, and the introduction of contaminants during the extraction and processing of harvested rainwater are possible sources of microbial contamination of rainwater this view was also asserted by (Ahmed *et al*., 2012a; Kaushik *et al*., 2014; Schets *et al*., 2010).

*Escherichia coli* (*E. coli*) is a facultative anaerobic bacterium that lives in the large intestines of warm-blooded mammals and is a significant member of the typical flora of the human colon (Thursby, 2017). Thus, the presence of *E. coli* in food or water often indicates recent faecal contamination or inadequate sanitary conditions in food or water processing plants (Odonkor and Ampofo, 2013). As a result, the population of *E. coli* is greatly influenced by faecal contamination, inadequate sanitation practices, and poor storage conditions (Agensi *et al*., 2019; Kayembe *et al*., 2018). The presence of *E. coli* in water does not automatically indicate the presence of pathogenic bacteria. However, it provides evidence of the potential presence of faecal-borne bacteria (Price and Wildeboer, 2017; Brussow, 2005). This explains why food and water samples are examined for *E. coli* in order to determine the levels of fecal contamination (Price and Wildeboer, 2017). Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Hudault *et al.,* 2011; Vogt and Dippold, 2015). The relationship between rainwater harvesting and the increase in *E. coli* related diseases in Otuoke community is currently not present in existing literature. This study is limited to Azikel road, hospital Road and Federal University West Campus, Otuoke in Ogbia Local Government of Bayelsa State.

### Study Area

Otuoke community is located at 4°42'23.418"N and 6°19'44.472E. Otuoke community is a suburban area located within the Ogbia Local Government Area of Bayelsa State, Nigeria. The majority of its residents are engaged in agricultural and fishing activities. The wet season is warm and overcast. The wet season usually starts from March to October, with a dip in August. The dry season is hot and partly cloudy. Over the course of the year, the temperature typically varies from 22°C to 34°C and is rarely below 18°C or above 40°C.

**Table 1: location of the sample sites**

| Location site | GPS Reading |
|---|---|
| Federal University Otuoke | 4º48’3.294” N<br>6º18’54.67788” E |
| Hospital Road | 4º47’31.96212” N<br>6º19’1.92612” E |
| Azikiel Road | 4º47’30.75612” N<br>6º19’8.24988” E |

**Sample Collection**

Samples used for the research work were rain water collected from three (3) different locations in Otuoke community namely Azikel Road, Hospital Road and Federal University Otuoke West Campus. The water samples were collected from rain water reservoirs, and a control gotten directly from the rain. They were collected into sterile sample bottles, and transported to the laboratory for analysis.

**Preparation of Nutrient Agar (NA):**

28 grams of the medium was dissolved in 1000ml of distilled water. It was heated, to dissolve the powder. Sterilization was done by autoclaving at 121°C for 15 minutes and the medium was dispensed into sterile Petri dishes and left to solidify, for inoculation.

**Preparation of Manitol Salt Agar (MSA):**

111.02 grams of the Mannitol Salt Agar was suspended in 1000 ml of distilled water (contained in a conical flask), the medium was heated, to dissolve completely. The resulting suspension was sterilized by autoclaving using an autoclave, at 15 lbs pressure (121°C) for 15 minutes. It was allowed to cool to 50°C and poured into sterile Petri dishes, to solidify.

**Preparation of Salmonella Shigella Agar (SSA):**

63grams of the medium was added into one liter (1000ml) of distilled water. The medium was properly mixed, and heated with frequent agitation and boiled for one minute. At the end, the medium was aseptically poured into sterile Petri dishes, and allowed to solidify.

**Preparation of Eosin Methylene Blue (EMB) Agar:**

36grams of the medium was suspended in 1000 ml distilled water, and mixed until the suspension was uniform. It was further heated to boiling to dissolve the medium completely, and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The medium was allowed to cool to 45-50°C and agitated in order to oxidize the methylene blue, and to suspend the flocculent precipitate. It was then poured into sterile Petri dishes. The plates were allowed to solidify, and warm to room temperature, before inoculation.

**Preparation of Simmons Citrate Agar (for Citrate Test):**

Simmons Citrate Agar is an agar medium used for the differentiation of Enterobacteriaceae based on the utilization of citrate as the sole source of carbon. 5.0 ml of the suspended medium was dispensed into 12-mm test tubes, and autoclaved at 121°C under 15 psi pressure (for 15 minutes). The tubes were Left to cool in slanted position.

**Preparation of Tryptophan Broth, MR/VP Broth**

5.0 ml of the suspended medium was dispensed into 12-mm test tubes, and autoclaved at 121°C under 15 psi pressure (for 15 minutes).

**Procedure for the enumeration of bacteria in the water samples:**

The process involved Serial Dilution, Inoculation, Incubation, Subculturing, Isolation, Monitoring, and Identification.

**Serial Dilution:**

10 fold serial dilution was used for the enumeration process. 10 12ml test tubes containing 9ml each of distilled water for each of the samples were sterilized using autoclave at 121°C psi for 15 minutes.

**Enumeration and Isolation of Total Bacteria**

The samples were serially diluted using 10 fold serial dilution, and 0.1ml of each diluted sample from 10−5 diluent was plated onto the nutrient agar, and spread on the surface, using the spread plate technique. The plates were incubated at 37°C for 24-48 hours.

**Procedure for Biochemical Characterization**

**MR TEST:** Using organisms taken from a 24 hour old pure cultures, using a sterile wire loop, each bacteria isolate was inoculated into the prepared Methyl Red broth. The tubes were incubated at 37°C, for 24 hours. Following 24 hours of incubation, 2 to 3 drops of methyl red indicator was added to the tubes, and observed for red colouration immediately.

**VP TEST:** Using organisms taken from a 24 hour old pure cultures, using a sterile wire loop, each bacteria isolate was inoculated into the prepared Voges-Proskauer broth. The tubes were incubated at 37°C, for 24 hours. Following 24 hours of incubation, 1ml each of Barrett's reagents A & B was added to the tubes. The tube was shaken gently, and allowed to stand for 15-30 minutes.

**INDOLE TEST:** The 24 hour old isolates were added to the Tryptophan broth prepared in the test tubes and sterilized, with the help of a sterile wire loop, with a control tube and incubated for 24 hours. After that, 1ml of Kovac's indole reagent was added to all the tubes, including the control. The tubes were agitated gently, and results recorded after 10-15 minutes.
Citrate Test and Oxidase Test were also carried in tubes to observe for the presence or absence of blue colouration. Catalase Test was done to observe for the presence or absence of bubbles. While Coagulase Test was done to observe for the clumping or agglutination of the mixture over the period of 10-15 seconds.

**Gram's Reaction:** A thin smear of the isolates from 24hours old pure cultures were made on clean glass slides, and allowed to air dry. The slides were heat fixed, by passing them through a Bunsen burner flame.

Following the fixation process, the slides were covered with crystal violet, for 1minute, and washed with distilled water after that. They were covered with Gram's Iodine for another 1minute, and washed off again with distilled water. Decolonization with 95% ethanol was done for 10 seconds, followed by counter staining with saffranin for 40 second. The slides were allowed to air dry and observed under the microscope, using oil immersion lens.

## RESULTS

**Table 2: Biochemical Characterization/Gram's Reaction**

| ORGANISM TEST | Escherichia coli | Psuedomonas aeruginosa | Staphylococcus aureus |
|---|---|---|---|
| MR | + | - | + |
| VP | - | - | + |
| Indole | + | - | - |
| Citrate | - | + | + |
| Oxidase | - | + | - |
| Catalase | - | + | + |
| Coagulase | - | - | + |
| Gram's Reaction | - | - | + |

**Table 3: Results for the Isolates of Total heterotrophic bacteria**

| Sample | No. Of Colonies | CFU/ml |
|---|---|---|
| Azikel Road | 264 | $26.4x10^6$ CFU/ml |
| Hospital Road | 226 | $22.6x10^6$ CFU/ml |
| Inside School | 143 | $14.3x10^6$ CFU/ml |
| Sample Control | 26 | Insignificant |

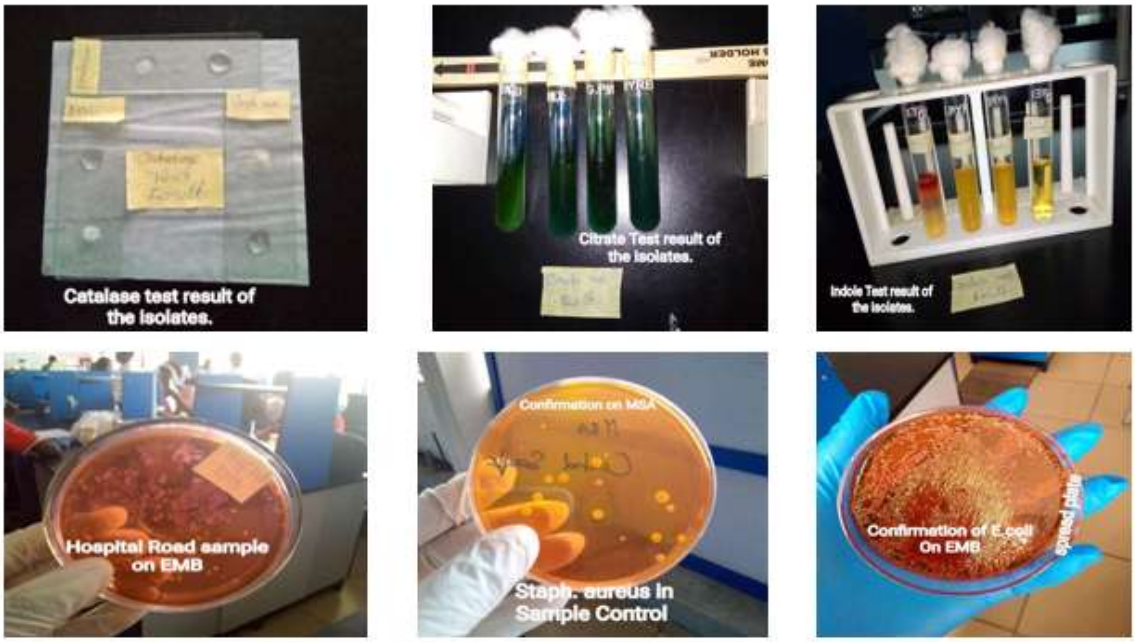


Figure 1: Biochemical Characterization and **Heterotrophic Bacteria** Test Results

After 48 hours of incubation, distinct colonies suspected (using culturing characteristics) to be *E. coli*, *Staph. aureus*, *Psuedomonas, Salmonella* etc were sub-cultured onto other special media.

EMB was used for *E. coli*, SSA was used for Salmonella, MSA was used for *Staph. aureus*.

Three (3) of the samples were positive for *E. coli* with Azikel Road sample having the highest microbial load, followed by Hospital Road Federal University

Otuoke (FUO), carrying the lowest. For Salmonella, all of the samples had Negative results. However, other opportunistic pathogens such as *Psuedomonas aeruginosa, Staphylococcus aureus,* and yeast were present in all of the samples, including the control sample (though in insignificant number, for the control sample). For these other bacteria isolates, Hospital Road sample had the highest levels of Staph and Psuedomonas, followed by Azikel Road sample, FUO still had the least microbial load. The control sample had few (insignificant) colonies of *Psuedomonas aeruginosa, Staph. aureus*, and yeast, which could be as a result of condensation from suspended dust in the air.

Purple colonies with green metallic sheen, (on EMB,) colourless colonies with hemolysis on SSA, Yellow colonies with fermentation on MSA, and light green-blue colonies on NA were further sub-cultured back onto Nutrient Agar at 37°C for 24 hours, for biochemical characterization.

## DISCUSSION

The rainwater samples were taken from three locations namely Azikiel road, Hospital Road and within the Federal University Otuoke premises. The highest bacteria load was at Azikiel road and it is followed by the samples gotten from Hospital Road. The control sample had insignificant bacteria load recorded. The micro-organisms that were detected include *E. coli*, *Salmonella*, *Pseudomonas* and *Staphylococcus aureus*. The Gram's reaction indicates the Gram staining characteristics of the organisms. All three organisms are Gram-negative. Three (3) of the samples were positive for *E. coli* with Azikel road sample having the highest microbial load, followed by Hospital Road, and samples from within the school premises, carried the lowest. For Salmonella, all of the samples had negative results.

However, other opportunistic pathogens such as *Psuedomonas aeruginosa, Staphylococcus aureus*, and yeast were present in all of the samples, including the control sample (though in insignificant number, for the control sample). For these other bacteria isolates, Hospital Road sample had the highest levels of *Staphylococcus aureus* and *Psuedomona*s, followed by samples from Azikel Road sample, and within the university premises still had the least microbial load. The Control sample had few (insignificant) colonies *of Psuedomonas aeruginosa, Staphylococcus aureus*, and yeast, which could be as a result of condensation from suspended dust in the air. A study by Ahmed *et al*., (2010) showed a list of pathogens that were isolated from rain water harvesting which includes *Salmonella spp, G. lamblia and L. pneumophila.* Rainwater tanks can face contamination as debris is washed into them from the roof and gutters during rain events. The main contributors to pathogen presence are expected to be fecal materials from birds, lizards, and possums that have access to the roof. *Salmonella* spp. has previously been detected in roof-harvested rainwater cisterns and in tanks as reported by Simmons *et al*., (2001).

## CONCLUSION

This study focused on rainwater harvesting for domestic use in Otuoke community and it also looks at its role in the prevalence of *Escherichia coli* related diseases and other water borne pathogenic microorganisms. *Escherichia coli* is generally employed as an indicator of faecal pollution by warm-blooded animals and there is a general high frequency of detection of these indicator organisms in rain water samples gotten from rooftops resulting in the contamination of the rainwater with the faecal matter from animals, amongst others, that might be deposited on the rooftops or in the gutter systems. *Escherichia coli*, a member of faecal coliforms has a significant place in water microbiology as an indicator of faecal pollution and a pathogen in drinking water. As a pathogen, it causes a variety of diseases ranging from urinary tract infections, sepsis, meningitis and bacteraemia to diarrhoea. *Escherichia coli* poses a health risk to rainwater from rooftop end-users in Otuoke and other communities with similar environmental characteristics, especially when used for drinking, washing kitchen utensils, cleaning of the home, garden hosing, washing laundry by hand, or when accidentally consumed by human beings. This study results show the presence of different pathogenic bacteria in the rainwater samples gotten from the study area which indicates a strong relationship between rainwater consumption within the study area and the occurrence of *E. coli* related diseases. Hence, a comprehensive approach for safe and effective rainwater utilization should be implemented. Community workshops and awareness campaigns should be organized to educate residents about the benefits and potential risks associated with rainwater harvesting. Regular testing for microbial contaminants and treatment of such, especially *Escherichia coli*, will ensure that the harvested rainwater remains a safe and reliable source for domestic use.

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**Cite this Article:** Onu, B (2024). The Role of Rain Water Harvesting for Domestic Use in the Prevalence of *Escherichia coli* (E. coli) Diseases in Otuoke Community. *Greener Journal of Environmental Management and Public Safety*, 12(1): 16-21.