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ANTIMICROBIAL SUSCEPTIBILITY AND BIOFILM FORMATION PROFILES OF Escherichia coli ISOLATES IN STORED DRINKING WATER FROM THREE COMMUNITIES IN EGOR LOCAL GOVERNMENT AREA, EDO STATE, NIGERIA

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ABSTRACT

Access to clean and safe drinking water remains a significant challenge in many rural areas, with improper storage and contamination posing serious risks to public health. This study examines various factors influencing the quality of stored drinking water, including sociodemographic characteristics of households, water sources, storage methods, antimicrobial susceptibility and biofilm formation profiles of *Escherichia* coli isolates in stored drinking water from Ugbiyokho, Evbuotubu and Iguediayi Communities, Egor Local Government Area, Edo State. Nigeria. A combination of classical IMVIC tests and the API 20E system was used for the identification of *E. coli*, allowing for accurate differentiation from other potentially similar bacteria. Biofilm formation was assessed qualitatively by the tube adherence method and quantitatively by the microtiter plate assay. The research utilized a structured questionnaire (*n*=400) to collect data on sociodemographic characteristics, water sources, types of storage systems, and the duration of water storage from Ugbiyokho, Evbuotubu, and Iguediayi communities in Egor Local Government, Edo State Nigeria. Among the 118 isolates tested, 66(55.9%) were resistant to ampicillin, while 39(78.1%) displayed resistance to piperacillin. Conversely, combination agents such as amoxicillin-clavulanate and ampicillin-sulbactam demonstrated high sensitivity rates, with 105(88.9%) and 110(93.2%) of isolates susceptible, respectively. No resistance was detected to carbapenems, as all isolates were fully sensitive to imipenem. Among fluoroquinolones, ciprofloxacin showed moderate susceptibility with 72(61%) of isolates sensitive, while 18(15.3%) were resistant. Macrolides, nitrofurans, and fosfomycin displayed strong effectiveness, with susceptibility rates of 95(80.5%), 95(80.5%), and 107(90.7%), respectively. Notably, cephems such as cefotaxime and ceftazidime also exhibited high efficacy, with 107(90.7%) and 110(93.2%) sensitivity, respectively. The tube adherence assay categorized 41(34.7%) of isolates as non-biofilm producers, while 17(14.4%) were weak biofilm producers, 36(30.5%) were moderate biofilm producers, and 24(20.3%) were strong biofilm producers. The microtiter plate assay provided a similar distribution, with 45(38.1%) classified as non-biofilm producers, 13(11%) as weak biofilm producers, 38(32.2%) as moderate biofilm producers, and 22(18.6%) as strong biofilm producers. These results demonstrate that a notable proportion of the isolates had significant biofilm-forming abilities, which could enhance their survival and resistance in hostile environments. The combination of high antimicrobial resistance and biofilm-forming ability among *E. coli* isolates from household water supplies poses a significant public health threat. These bacteria can persist in stored water, evade treatment, and cause infections that are difficult to manage with commonly available antibiotics. The results highlights the urgent need for improved water treatment and storage practices; regular monitoring of antimicrobial resistance in environmental isolates; public health education on the risks of antibiotic misuse and the importance of safe water handling.

KEYWORDS: Antimicrobial, Profile, Biofilm, Water, Community.

INTRODUCTION

Access to safe drinking water is a basic human right essential for health and development (UN-Water Decade Programme on Advocacy and Communication, 2011). However, water contamination poses a major public health threat globally, particularly in low- and middle-income countries with limited access to safe water (UN-Water, 2021). According to the WHO, nearly 2 billion people worldwide use drinking water contaminated with faecal matter, resulting in widespread waterborne diseases (Agensi *et al*., 2019). Diarrheal diseases linked to unsafe water and poor sanitation cause over half a million deaths annually, mostly in children under five (WHO/UNICEF, 2010; Abu and Codjoe, 2018). Ensuring safe drinking water is thus a critical global priority, especially in vulnerable regions with inadequate infrastructure.

In rural Africa, including Nigeria, water quality issues are compounded by poverty, inadequate water infrastructure, and poor sanitation (Isukuru *et al*., 2024). Even water from relatively safe sources like boreholes or protected wells can become contaminated due to improper household storage and handling (Genter *et al*., 2021). This highlights the importance of assessing both water sources and household storage practices, as the latter often increases contamination risks significantly. Nigeria, Africa's most populous nation, faces major challenges in providing clean and safe drinking water, particularly in rural areas (Isukuru *et al*., 2024). Despite some improvements in urban water infrastructure, about 57 million Nigerians still depend on unimproved or unsafe water sources (USAID/Nigeria, 2017). Reports from the Nigerian National Bureau of Statistics and UNICEF highlight the prevalence of waterborne diseases like diarrhoea, cholera, and typhoid in rural communities where access to safe water and proper sanitation is lacking (Federal Ministry of Water Resources, Government of Nigeria, National Bureau of Statistics and UNICEF, 2020). In these areas, municipal water supplies are rare, and people often rely on untreated surface water, rivers, shallow wells, and boreholes.

In rural Nigeria, inadequate household water storage practices worsen water quality problems (Okoh *et al*., 2021). Many rural families collect and store water for days in poorly maintained containers, promoting the growth of pathogens, especially faecal coliforms like *E. coli*. This combination of unreliable water sources and substandard storage conditions heightens the risk of microbial contamination, potentially causing serious health issues. Egor Local Government Area in Edo State, Nigeria, faces significant water quality challenges typical of many rural Nigerian communities. Residents primarily rely on untreated and unmonitored water sources like boreholes, wells, and streams. Seasonal rains exacerbate contamination through agricultural and human waste runoff (Bolan *et al*., 2024). This situation puts rural households at high risk of exposure to unsafe water, with additional contamination risks during transportation and household storage. Water storage in rural communities is a frequently overlooked source of contamination (Cassivi *et al*., 2021). Irregular water access leads to prolonged storage in large containers, creating ideal conditions for microbial growth (Salehi, 2022). Poor container maintenance, exposure to air, and improper sealing can contaminate even initially safe water (Alamri *et al*., 2021). In low-resource areas with unregulated antibiotic use, water can become a conduit for resistant bacteria (Bebell and Muiru, 2014). Analysing AMR profiles in *E. coli* from household-stored water offers crucial insights into the local AMR burden and helps shape strategies to curb the spread of resistant strains Biofilms enhance bacterial resistance to environmental stressors and antibiotics, complicating pathogen elimination from water supplies (Dutt *et al*., 2022). This study aims at determining the antimicrobial susceptibility and biofilm formation profiles of *E. coli* isolates in stored drinking water in three rural communities.

MATERIALS AND METHOD

Area of Study

Geographical Description of Study Area

The study was conducted in rural communities within Egor Local Government Area, Edo South, Nigeria. Egor LGA, located in the southern part of Nigeria, is predominantly rural, with many households relying on stored water for daily consumption due to irregular water supply. The study adopted a cross-sectional design, which allowed for the collection of data from different households at a single point in time to assess the quality of household-stored drinking water. The study was designed to assess the physicochemical and microbiological parameters of water stored in households, as well as the associated risk factors for contamination.

Study Population and Sample Size

The target population included households from rural communities within Egor LGA (Ugbiyokho, Evbuotubu and Iguediayi) that rely on stored drinking water. A multi-stage sampling technique was employed to ensure the study's sample was representative of the population. First, the communities were stratified based on geographic location, dividing them into sub-regions within Egor LGA. Second, a random selection of households within each stratum was conducted. The selection criteria for households included the presence of stored drinking water, either for daily consumption or for longer-term use, and consent from household members to participate in the study. The sample size was determined using a statistical formula for cross-sectional studies based on an estimated prevalence of water contamination of 50%, a confidence level of 95%, and a margin of error of 5%.

$$Sample\ size = Z^2 P(1-P)/C^2$$

Where Z = Z value (1.96 for 95 % confidence level); P = expected prevalence; C = confidence interval (0.05) (Pant *et al*., 2016). An additional 10% was added to account for non-responses or incomplete data, resulting in a final sample size of approximately 300 households. However, a total of 400 stored drinking water samples were collected.

Ethical Considerations

Ethical approval for the study was obtained from the Ethics Committee of Life Sciences, University of Benin. Written informed consent was obtained from all participating households prior to data collection. All water samples and household data were anonymized to protect the privacy of participants, and the study adhered to ethical guidelines on human subject research.

Data Collection Instruments and Procedures

A structured questionnaire was developed and administered to collect information on the sociodemographic characteristics of households, the source of water, type of storage system, and duration of storage. The questionnaire was designed to gather comprehensive information about household water management practices and perceptions in the rural communities of Egor Local Government Area. It began by collecting demographic data, including the age, gender, education level, and occupation of household members, to provide context for the subsequent responses. Water samples were collected from each participating household from rural communities (Ugbiyokho n=140, Evbuotubu n=130, and Iguediayi n=130) in different households from Egor Local Area, Edo State Nigeria, using 150 ml sterile containers to avoid contamination during the sampling process. Samples were taken directly from the stored water containers in the households, ensuring that representative samples were collected from different types of storage systems. Samples were transported to the laboratory within 2 h of collection in ice-packed coolers maintained at a temperature between 2°C to 8°C to the Applied Microbial Processes & Environmental Health Research (AMPEHREG) laboratory for analysis. A total of 400 water samples were collected, labeled, and assigned unique identification codes corresponding to the households.

Statistical Analysis

Data collected from the structured questionnaires, sanitary inspections, and laboratory tests were analyzed using Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics such as means, medians, frequencies, and percentages were used to summarize the sociodemographic characteristics of households, water storage practices, and sanitary risk scores. Inferential statistics, including t-tests, were employed to assess the relationship between water quality parameters and household practices. Multivariate correlation analysis was used to identify factors significantly associated with microbial contamination.

RESULTS

The study conducted in rural communities of Egor Local Government Area, Edo South, Nigeria, revealed diverse demographic characteristics among respondents. The age distribution showed that the majority of participants were between 31-45 years old, comprising 46% of the sample, followed by those aged 18-30 (25.3%), 46-60 (22.3%), and over 60 (6.5%). Women made up a larger portion of the respondents at 251 (62.8%), compared to 149 (37.3%) men (Table 1). In terms of education, more than half of the participants 212 (53%) had primary education, while 123 (30.8%) completed secondary education, and 65 (16.3%) attained tertiary education (Table 1). The occupational breakdown indicated a diverse range of livelihoods, with students forming the largest group at 72 (18%), followed by those engaged in trading 57 (14.3%) and agriculture 56 (14%). Other occupations included bricklayers, civil servants, engineers, teachers, and various skilled trades (Table 1). The study covered three communities, with Iguediayi having the highest representation at 167 (41.8%), followed by Ugbiyokho at 138 (34.5%), and Evbuotubu at 115 (28.8%). The study revealed several key findings regarding water storage practices. The majority of respondents, 212 (53%), used plastic containers for storing drinking water, while 167 (41.8%) used metal containers, and only 21 (5.2%) used clay pots (Table 2). In terms of cleaning frequency, 231 (57.8%) of participants reported cleaning their water storage containers weekly, 79 (19.8%) cleaned monthly, 71 (17.8%) cleaned rarely, and a small percentage of 19 (4.8%) cleaned daily (Table 2). Notably, all respondents 400 (100%) reported covering their water storage containers. The antimicrobial susceptibility profile of the *E. coli* isolates revealed significant variability across different antibiotic classes. Among the 118 isolates tested, high levels of resistance were noted for certain antibiotics, particularly within the penicillin class. For example, 66(55.9%) of isolates were resistant to ampicillin, while 39(78.1%) displayed resistance to piperacillin (Table 3). Conversely, combination agents such as amoxicillin-clavulanate and ampicillin-sulbactam demonstrated high sensitivity rates, with 105(88.9%) and 110(93.2%) of isolates susceptible, respectively. No resistance was detected to carbapenems, as all isolates were fully sensitive to imipenem. Among fluoroquinolones, ciprofloxacin showed moderate susceptibility with 72(61%) of isolates sensitive, while 18(15.3%) were resistant. Macrolides, nitrofurans, and fosfomycin displayed strong effectiveness, with susceptibility rates of 95(80.5%), 95(80.5%), and 107(90.7%), respectively. Notably, cephems such as cefotaxime and ceftazidime also exhibited high efficacy, with 107(90.7%) and 110(93.2%) sensitivity, respectively (Table 3).

Biofilm formation was assessed using two methods: tube adherence and microtiter plate assays. The tube adherence assay categorized 41(34.7%) of isolates as non-biofilm producers, while 17(14.4%) were weak biofilm producers, 36(30.5%) were moderate biofilm producers, and 24(20.3%) were strong biofilm producers (Figure 1). The microtiter plate assay provided a similar distribution, with 45(38.1%) classified as non-biofilm producers, 13(11%) as weak biofilm producers, 38(32.2%) as moderate biofilm producers, and 22(18.6%) as strong biofilm producers (Figure 2). These results demonstrate that a notable proportion of the isolates had significant biofilm-forming abilities, which could enhance their survival and resistance in hostile environments.

Table 1: Demographic characteristics among respondents.

Variable		Number (n=400)	Percentage
Age	18-30	101	25.3
	31-45	184	46.0
	46-60	89	22.3
	>60	26	6.5
Gender	Male	149	37.3
	Female	251	62.8
Education level	Primary	212	53.0
	Secondary	123	30.8
	Tertiary	65	16.3
Occupation of household members	Trading	57	14.3
	Agriculture	56	14.0
	Civil Servant	38	9.5
	Banker	7	1.8
	Engineer	34	8.5
	Bricklayer	49	12.3
	Vulcanizer	6	1.5
	Electrician	11	2.8
	Transporter	18	4.5
	Tiler	5	1.3
	Plumber	9	2.3
	Teacher	27	6.8
	Welder	11	2.8
	Student	72	18.0
Village/Community name	Ugbiyokho	138	34.5
	Evbuotubu	115	28.8
	Iguediayi	167	41.8

Table 2: Water storage practices among respondents.

Water Storage Practices questions		Number (n=400)	Percentage
What type of container do you use to store drinking water?	Clay pot	21	5.2
	Plastic container	212	53.0
	Metal container	167	41.8
How often do you clean your water storage container?	Daily	19	4.8
	Weekly	231	57.8
	Monthly	79	19.8
	Rarely	71	17.8
Do you cover your water storage container?	Yes	400	100
	No	0	0
How do you usually collect water from the storage container?	Dipping cup/ladle	281	70.3
	Pouring	119	29.8
How long do you typically store water before use?	Less than 1 day	183	45.8
	1-3 days	198	49.5
	4-7 days	7	1.8
	More than 1 week	12	3.0

Table 3: Antimicrobial susceptibility profiles of the *E. coli* isolate.

Antimicrobial class	**Antibiotics**	**Susceptibility profile n=118 (%)**		
		Resistance	**Intermediate**	**Sensitive**
Penicillins	Ampicillin	66(55.9)	21(17.8)	31(26.3)
	Piperacillin	39(78.1)	35(29.7)	44(37.3)
β-lactam combination agents	Amoxicillin-clavulanate	5(4.2)	8(6.8)	105(88.9)
	Ampicillin-sulbactam	3(2.5)	5(4.2)	110(93.2)
Monobactams	Aztreonam	41(34.7)	12(10.2)	65(55.1)
Tetracyclines	Tetracycline	52(44.1)	31(26.3)	35(29.7)
Carbapenems	Imipenem	0	0	118(100)
Fluoroquinolones	Ciprofloxacin	18(15.3)	28(23.7)	72(61.0)
Macrolides	Azithromycin	12(10.2)	11(9.3)	95(80.5)
Nitrofurans	Nitrofurantoin	9(7.6)	14(11.9)	95(80.5)
Phenicols	Chloramphenicol	31(26.3)	29(24.6)	58(49.2)
Aminoglycosides	Gentamicin	9(7.6)	4(3.4)	105(88.9)
	Amikacin	7(5.9)	6(5.1)	105(88.9)
Folate pathway antagonists	Trimethoprim-sulfamethoxazole	29(24.6)	19(16.1)	70(59.3)
Fosfomycins	Fosfomycin	11(9.3)	0	107(90.7)
Cephems	Cefotaxime	5(4.2)	6(5.1)	107(90.7)
	Ceftazidime	4(3.4)	4(3.4)	110(93.2)

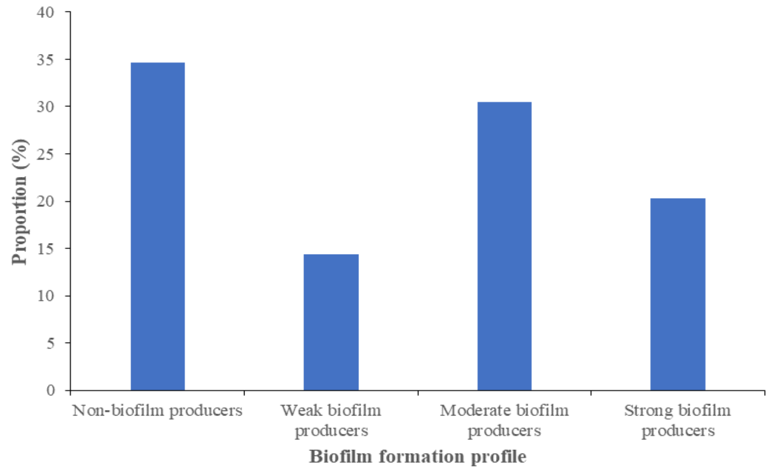

Figure 1: Tube adherence biofilm formation of the *E. coli* Isolates.

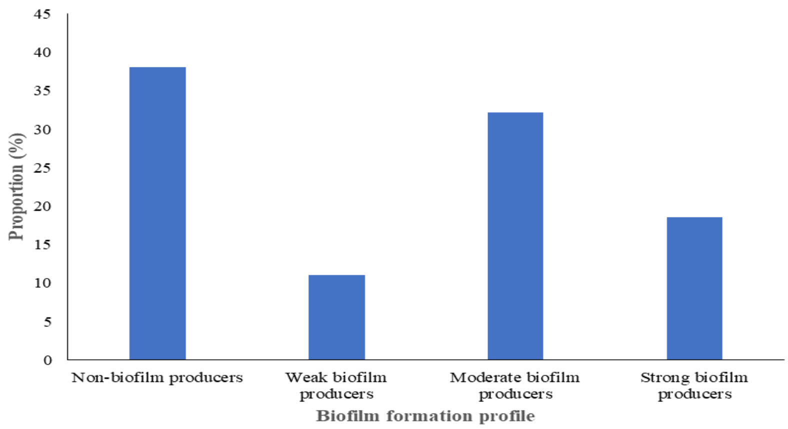

Figure 2: Microtiter plate biofilm formation of the *E. coli* Isolates.

DISCUSSION

The demographic characteristics observed among respondents in rural communities of Egor LGA, Edo State, Nigeria, reflect trends seen in similar studies across rural Nigeria. The dominance of the 31-45 age group mirrors findings from the 2018 Nigeria

Demographic and Health Survey (NDHS), which reported that the majority of rural populations are within the economically active age bracket (National Population Commission and ICF, 2019). The higher proportion of women among respondents is also consistent with rural survey trends, where women are often more available and willing to participate in community-based research (Ene-Obong *et al.*, 2017). This gender imbalance is frequently attributed to male migration for employment and traditional gender roles in rural Nigeria. The finding that over half of respondents had only primary education aligns with national statistics showing lower educational attainment in rural areas compared to urban centres (NPC and ICF, 2019). The NDHS reports that only about 13% of rural women and 21% of rural men have completed secondary education or higher, which is comparable to the 30.8% secondary and 16.3% tertiary attainment found in this study.

All water samples from the three communities failed to meet WHO microbiological standards, which require zero coliforms and *E. coli* per 100 mL for safe drinking water (WHO, 2017). The presence of total coliforms, faecal coliforms, and *E. coli* indicates faecal contamination and inadequate water treatment. These findings are consistent with other Nigerian and sub-Saharan African studies, where stored household water frequently shows microbial contamination due to poor source protection, inadequate storage, and unsafe handling (Alabi *et al.*, 2024; Tenebe *et al.*, 2024). The detection of *E. coli*, a reliable indicator of faecal pollution, underscores the risk of waterborne diseases. The use of classical biochemical tests, API 20E, and PCR for *E. coli* identification enhances the reliability of the findings.

The antimicrobial susceptibility testing of *E. coli* isolates from household-stored drinking water in the three communities revealed a concerning pattern of resistance, particularly to penicillins. Over half of the isolates were resistant to ampicillin and an even higher proportion to piperacillin. This is consistent with findings from other studies in Nigeria and globally, where high rates of resistance to penicillins among environmental and clinical *E. coli* isolates are commonly reported (Singh *et al.*, 2020; Titilawo *et al.*, 2023). However, the high susceptibility to β-lactam/β-lactamase inhibitor combinations and to carbapenems is encouraging and aligns with broader surveillance data (WHO GLASS Report, 2022). These findings suggest that while first-line antibiotics are increasingly compromised, more advanced agents retain efficacy, though their use in community settings is limited by cost and availability. High susceptibility to macrolides, nitrofurans, and fosfomycin is consistent with their lower usage in the region and their effectiveness against environmental isolates (Chigor *et al.*, 2020). Biofilm formation assays revealed that a considerable fraction of isolates was moderate to strong biofilm producers. Biofilm formation enhances bacterial survival in adverse environments, increases resistance to disinfectants and antibiotics, and is associated with chronic and recurrent infections (Sharma *et al.*, 2023). The observed rates are comparable to those reported in other water and clinical studies in Africa and elsewhere (Rabiu *et al.*, 2022; Bello *et al.*, 2024; Oyewale *et al.*, 2024), underscoring the persistence and adaptability of *E. coli* in water storage systems. The combination of high antimicrobial resistance, diverse virulence factors, and biofilm-forming ability among *E. coli* isolates from household water supplies poses a significant public health threat. These bacteria can persist in stored water, evade treatment, and cause infections that are difficult to manage with commonly available antibiotics. This highlights the urgent need for improved water treatment and storage practices; regular monitoring of antimicrobial resistance in environmental isolates; public health education on the risks of antibiotic misuse and the importance of safe water handling.

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