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# Comparative Study on Water Quality Monitoring of Underground and Surface Water in Some Rural Agricultural Communities in Ogun State, South-Western Nigeria

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**ABSTRACT:** Portable water supply is crucial to the survival of the human race since water is life. Samples of water from 69 wells, 40 boreholes and 4 streams were collected and the isolation of microorganisms from the samples was carried out according to International Standard Organization (ISO) methods and bacteria recovered were characterized using standard biochemical tests. The susceptibility of the bacteria to antibiotics was ascertained using the Kirby-Bauer disc diffusion method. The highest viable, coliform, *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas* and fungal counts were 1683.33 $\pm$ 416.67, 52.67 $\pm$ 6.26, 6.50 $\pm$ 1.50, 2.00 $\pm$ 0.00, 1.50 $\pm$ 0.0, 4.00 $\pm$ 0.00, 10.00 $\pm$ 2.35 from Ilaporu wells, Mamu wells, Mamu stream, Mamu stream and wells, Mamu stream, Mamu boreholes and Oru stream, respectively. The occurrence of the bacteria in descending order was *Klebsiella pneumoniae* (31.7%), *Enterobacter* spp (27.0%), *E. coli* (20.4%), *P. aeruginosa* (9.0%), *Salmonella* sp (8.4%), *Shigella* sp. (3.0%) and *Proteus mirabilis* (0.6%). Fungi such as *Penicillium, Aspergillus* and *Trichoderma* were recovered. The susceptibility of the bacteria to the different antibiotics showed 72.5% susceptibility to ciprofloxacin and 19.8% to chloramphenicol. The presence of *E. coli*, which is known as a faecal indicator of water quality in these water sources, calls for concern because consumption of this water may lead to waterborne diseases.

Keywords: Antibiotics resistance, Bacteria, Community, Fungi, Water

#### Introduction

Water is indispensable for the survival of all organisms. Without water, there is no life as plants depend on water for growth, while animals feeding on these plants also need water for digestion. Access to potable water is crucial to human survival; hence, the absence of potable drinking water can subject people to serious waterborne diseases such as typhoid, gastroenteritis, cholera, amoebiasis, hepatitis, scabies and worm infections. About 2 billion people globally lack access to clean and safe drinking water (Bayram, 2023). There is a gradual increase in the contamination of river waters by microorganisms which include bacteria, parasites, and fungi (Niyogi, 2005; Abraham *et al.*, 2010) from humans and other animals. Also, water can be polluted by pathogens through the discharge of untreated sewage from wastewater plants (Donovan *et al.*, 2008; Musyoki *et al.*, 2013). Some of the pathogens include *Shigella*, *Escherichia coli*, *Salmonella*, *Vibrio*, *Norwalk* virus, *Entamoeba*, *Cryptosporidium* and *Giardia*.

The presence of fungi in surface or underground water has been reported by different authors, and the most frequently encountered genera are *Aspergillus, Penicillium* and *Acremonium* (Oliveria *et al.*, 2016; Nwankwo *et al.*, 2020; Ren *et al.*, 2023). Fungi are known for the production of odor and pigment in water as well as the blocking of water pipes (Hussain *et al.*, 2010). Among the yeasts, *Candida, Rhodotorula* and *Cryptococcus* have been reported (Pereira *et al.*, 2009; Ayanbimpe *et al.*, 2012).

Antibiotics are life savers, but recently microorganisms have become resistant to these antibiotics at an alarming rate. Antimicrobial Resistance (AMR) is identified as one of the threats to global health as bacteria resistant to antimicrobials will be the leading cause of death globally by 2050 (O'Neill, 2014). Human can be exposed to Environmental AMR bacteria through contaminated water and food which can result in infections. Most people living in agricultural settlements do not have access to clean and safe drinking water, rather, they get their water from unsafe sources such as streams. Some of these settlers defecate directly into the water bodies and still use the same water for domestic purposes. Unsafe drinking water transmits waterborne diseases to the population. Therefore, this research aims to compare the microbial load and identify the different microorganisms in the water samples to observe the quality of underground and surface water in rural agricultural settlements.

#### Materials and methods

Sampling: This cross-sectional study involved 69 hands dug wells, 40 boreholes and 4 streams across the study communities. The study communities are: Ago-Iwoye, Ilaporu, Oru, Awa, Ijebu-Igbo, Mamu and Abeokuta. Early morning visits were made to the communities and the Global Positioning System (GPS) coordinates of all the different sources of water were taken. Three (3) litres of water were collected from each source into polyethylene water containers. All the collected water samples were labelled, preserved in ice boxes at a temperature between 4 - 10 °C to avoid any contamination, and transported to the Microbiology Laboratory of the Department of Microbiology, Olabisi Onabanjo University, Ago - Iwoye for microbial analysis within the first 12 h.



Figure 1: Distribution of water samples and their sources among the communities visited within the study area

*Isolation of microorganisms:* Total viable count (TVC) (by the use of the spread plate method), total coliform count (TCC) and *Escherichia coli* count were carried out according to ISO (2000). The *P. aeruginosa* parameter was tested using the membrane filtration technique according to the standard method ISO 16266:2006 (ISO, 2006). Deoxycholate Citrate Agar (DCA) plates were streaked with the water samples and the plates were incubated at 37 °C for 24 h. For the detection of *Shigella* and *Salmonella*, the water samples were cultured on *Salmonella-Shigella* Agar.

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For fungi detection, 0.1 ml of each water sample was streaked on Malt agar and Potato Dextrose Agar (PDA) plates, and the plates containing were incubated at 25 - 30 °C. After 24 - 72 h, the number of mold and yeast colonies was counted using the colony counter (Rompre *et al.*, 2002).

*Identification of bacteria isolates:* Pure culture of positive colonies from all the media used were further identified using colonial, microscopic and biochemical tests. Catalase, indole, urease, citrate, oxidase, methyl red, triple sugar iron agar and sugar fermentation tests are the biochemical tests carried out as described by Cheesbrough (2006).

*Fungal identification:* Fungal colonies on PDA plates were subcultured several times, to get a pure culture of each isolate. Identification of fungi was done by observing the morphology under the microscope. The hyphae were stained with cotton blue in lactophenol, viewed under the x 40 objective lens and further identified with standard reference (Barnett and Hunter, 1987).

Antimicrobial susceptibility test: Antimicrobial susceptibility tests for the bacteria isolates recovered from the water samples were carried out using the Kirby-Bauer disc diffusion method (Bauer *et al.*,1966). The pure culture of the isolates was introduced into normal saline water and standardized to 0.5 McFarland solution. A sterile swab stick was used to seed the bacteria on Muller Hinton plates and the antibiotic discs: aztreonam (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), ceftazidime (30  $\mu$ g) and chloramphenicol (30  $\mu$ g) were placed on the plates aseptically. The plates were incubated invertedly at 35 °C overnight. The zones of inhibition of the antibiotic discs were read to the nearest millimeter (mm).

### Results

*Microbial loads in water sample*: The frequency of occurrence of bacteria in wells, boreholes and stream water from the study area collection sites is presented in Fig 1. Most of the bacteria recovered from the water samples were from Ago-Iwoye, followed by Abeokuta while Ilaporu had the least bacterial isolates.

The microbial load of the microorganisms in the water samples is presented in Tables 1a and 1b. The highest total viable count was from the Ilaporu well, followed by Oru stream; for the total coliform count, the Mamu well and borehole have the highest count of  $52.67\pm6.26^{a}$  and  $35.00\pm0.00^{b}$  respectively. The highest fungal load was from the Ago-Iwoye borehole, followed by water sourced from boreholes in Abeokuta and the least were from wells from Oru. Yeast and mould were not recovered from Oru and Awa boreholes and wells.

*Identification of microorganisms*: The colonial morphology of the bacteria isolates on the different media is presented in Table 2. One hundred and sixty-seven (167) Gram-negative bacteria were recovered from the water samples after carrying out Gram staining and biochemical tests, which comprised six genera of bacteria. The bacteria identified with their percentage occurrence were *Klebsiella pneumoniae* (31.7%), *Enterobacter* spp (27.0%), *E. coli* (20.4%), *P. aeruginosa* (9.0%), *Salmonella* sp (8.4%), *Shigella* sp. (3.0%) and *Proteus mirabilis* (0.6%) (Figure 3) while *Penicillium*, *Aspergillus*, *Trichoderma* and *Candida* were the fungi identified in the water.

Antimicrobial susceptibility test: The result of the antimicrobial susceptibility test is presented in Table 4. The susceptibility of the bacterial isolates in descending order to the tested antibiotics was 72.5%, 64.7%, 23.9% and 19.8% respectively for chloramphenicol, ceftazidime, aztreonam and ciprofloxacin. *Klebsiella* was the most sensitive to all the tested antibiotics.



Figure 2: Frequency of occurrence of bacteria in water sources in relation to the study area

	llaporu	Awa		Ago-lwoye	ljebu-lgbo						
	Well	Well	Borehole	Well	Borehole	Well	Borehole				
Microbial load in cfu/ml											
Total viable count,	1683.33±416.67ª	116.00±36.03°	325.83±110.80b	168.12±58.78 <sup>b</sup>	94.29±24.19b	286.00±92.44b	86.75±14.74 <sup>d</sup>				
cfu/ml											
Total coliform count	5.33±3.53 <sup>b</sup>	0.00±0.00°	4.83±3.17 <sup>b</sup>	3.83±1.71 <sup>₅</sup>	2.00±0.86 <sup>b</sup>	3.40±0.93 <sup>b</sup>	0.00±0.00 <sup>c</sup>				
E. coli cfu/100ml	0.33±0.33 <sup>b</sup>	0.00±0.00°	1.50±1.12 <sup>b</sup>	1.00±0.44 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.80±0.37 <sup>b</sup>	0.00±0.00°				
Salmonella count	0.00±0.00°	0.00±0.00°	0.33±0.33 <sup>b</sup>	0.58±0.42 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.20±0.20 <sup>b</sup>	0.00±0.00°				
Shigella count	0.00±0.00°	0.00±0.00°	0.17±0.17 <sup>b</sup>	0.25±0.18 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00°	0.00±0.00°				
P. aeruginosa count	1.33±0.67ª	0.00±0.00°	0.67±0.49 <sup>b</sup>	0.67±0.33 <sup>b</sup>	0.83±0.65 <sup>b</sup>	0.40±0.24 <sup>b</sup>	0.00±0.00 <sup>c</sup>				
Yeast & mould count	6.67±4.41ª	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	8.91±2.35 <sup>a</sup>	4.33±3.20 <sup>b</sup>	0.50±0.50 <sup>b</sup>	0.50±0.29°				
1 1											

 
 Table 1a: Microbial evaluation of drinking water collected from the rural communities of Ijebu-North, Southwestern Nigeria

 $^{abcd}$ Mean values (±Standard deviation) in the same row having similar superscripts are not significantly different (p > 0.05)

#### Table 1b: Microbial evaluation of drinking water collected from Mamu, Oru and Abeokuta, Ogun State Nigeria

	Mamu			Oru			Abeokuta	
	Stream	Well	Borehole	Stream	Well	Borehole	Well	Borehole
Total viable count	350.00±130.00ª	96.25±54.74 <sup>b</sup>	410.00±0.00ª	2100.00±0.0ª	148.8±25.18°	52.75±19.94 <sup>d</sup>	134.45±34.0℃	383.3±87.24 <sup>b</sup>
Total coliform count	25.00±10.00°	52.67±6.26ª	35.00±0.00 <sup>b</sup>	22.00±0.00 <sup>a</sup>	1.25±0.48℃	0.00±0.00 <sup>d</sup>	3.60±1.45℃	14.50±1.86 <sup>b</sup>
E. coli cfu/100ml	6.50±1.50ª	5.33 <b>±</b> 2.40ª	5.00±0.00ª	5.00±0.00ª	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.80±0.49 <sup>b</sup>	4.20±0.66 <sup>a</sup>
Salmonella count	2.00±0.00 <sup>a</sup>	2.00±0.00 <sup>a</sup>	1.00±0.00ª	4.00±0.00 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.60±0.43 <sup>b</sup>	1.60±0.40 <sup>b</sup>
Shigella count	1.50±0.50ª	1.00±0.00ª	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.20±0.13 <sup>b</sup>	1.00±0.26ª
P. aeruginosa	2.50±0.50 <sup>b</sup>	2.33±1.33 <sup>b</sup>	4.00±0.00ª	4.00±0.00 <sup>a</sup>	0.00±0.00°	0.00±0.00°	0.40±0.27℃	1.10±0.41 <sup>b</sup>
Yeast & mould count	7.00±3.00 <sup>a</sup>	4.67±2.91ª	5.00±0.00ª	10.00±0.00 <sup>a</sup>	0.25±0.25℃	0.00±0.00 <sup>c</sup>	4.60±3.01 <sup>b</sup>	7.70±1.42ª
abada a s								

<sup>abcd</sup>Mean values ( $\pm$ Standard deviation) in the same row having similar superscripts are not significantly different (p > 0.05)

No of isolates	Nutrient Agar	MacConkey Agar	Eosin Methylene Blue	Salmonella- Shigella Agar	Deoxycholate Agar
34	Large, thick, moist opaque, greyish white colony	Circular, convex, smooth, surface, pink, opaque	Circular, convex, smooth surface, green metallic sheen, opaque		
53	Large, mucoid, whitish colony	Large, mucoid, pink to red colonies	Large mucoid pink to purple colonies	Red coloured colonies	
15	Large, flat, opaque colonies with a greenish colour	Pink, mucoid small colonies	Pink colour colonies with a dark center		
45	U U	Round, flat and colourless colonies	Raised and mucoid with colourless colonies		
5	Grey, white, moist, smooth colonies		Colourless colonies	Smooth, colourless colonies with a black center	Colourless, smooth, and shiny colonies with black centers
14	Off-white colonies with smooth surface		Colourless colonies	Colourless colonies	Colourless, smooth, shiny colonies
1	Small, and glistening colony				-

# **Table 3:** Gram staining and biochemical characteristics of Gram-negative bacteria recovered from borehole, well and stream water samples

No of Isolates	Gram staining	Catalase	Oxidase	Citrate	Urease	Methyl red	۷P	Motility	Indole	Gas	H <sub>2</sub> S	Gelatin hydrolys	Starch	Sucrose	Glucose	Lactose	Arabinose	Mannitol	Maltose	Probable Identity
34	- R	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	-	E. coli
53	- R	+	-	+	+	-	+	-	-	+	-	-	-	+	+	+	+	+	+	Klebsiella pneumoniae
45	- R	+	-	+	-	-	+	+	-	+	-	-	-	+	+	+	+	+	+	Enterobacter aerogenes
15	- R	+	+	+	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	Pseudomonas aeruginosa
14	- R	+	-	-	-	+	-	+	-	-	+	-	nd	-	+	-	-	+	+	Salmonella typhi
5	- R	+	-	-	-	+	-	-	-	-	-	-	nd	-	+	-	-	+		Shigella flexneri
1	- R	+	-	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	Proteus mirabilis

Key: nd - not done, H2S - Hydrogen Sulphide, VP - Voges Proskauer, R - rods, - : negative, +: positive

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Figure 3: Rate of occurrence of Gram-negative bacteria in water samples

Isolate	Aztr	Aztreonam			ramp	henicol	Cef	tazid	ime	Cip	Ciprofloxacin		
code	c late	R	Ι	S	R	Ι	S	R	Ι	S	R	Ι	S
	No Isol												
KL	53	12	39	2	17	31	5	5	15	33	9	7	37
EA	45	10	20	15	9	29	7	5	11	29	9	6	30
EC	34	4	10	20	4	17	13	5	1	28	3	3	28
PA	15	6	8	1	2	5	8	4	4	7	4	0	11
ST	14	7	6	1	1	13	0	1	7	6	0	4	10
SF	5	0	5	0	0	5	0	0	1	4	0	1	4
PM	1	0	0	1	0	1	0	0	0	1	0	0	1
% total	167	39	88	40	33	10 1	33	20	39	108	25	21	121

Table 4: Classification of the bacterial isolates into susceptibility class

**Key:** KL - *Klebsiella pneumonie*, EA - *Enterobacter aerogenes*, EC - *Escherichia coli*, PA - *Pseudomonas aeruginosa*, ST - *Salmonella typhi*, SF - *Shigella flexneri*, PM - *Proteus mirabilis*, S - Susceptible, R - Resistant, I - Intermediate Resistant.

### Discussion

The total viable count of stream water in Oru is greater than the well and borehole while the microbial load for Mamu stream is lesser than the borehole but greater than the microbial load in the well. This conforms with the work of Shahina *et al.* (2020) who stated that the MPN indicator of the surface water obtained was higher when compared with groundwater. The total viable count was high in well water from Ilaporu and stream water from Oru while the highest total coliform count was recorded in Mamu well, closely followed by the borehole. WHO permissible limit for heterotrophic count in water is < 500 cfu/ml. Stream water from Oru and well water from Ilaporu exceeded this limit, therefore they are not fit for drinking. The presence of coliform in well water and underground water has been reported, such as Shahina *et al.* (2020) who reported the presence of a large number of coliforms in both surface and underground water. Coliform count in the water samples analyzed is greater than the permissible limit of WHO which says no *E. coli* must be detected in a 100 mL sample of drinking water (WHO, 2018), which means that all the drinking water samples containing coliform are not good for consumption.

The contamination of stream and well water from this area may be because of flood water entering into the stream while that of well water may be due to openness and shallowness of the groundwater that allows easy entrance of particles from the surrounding. It may also be a result of poor hygienic conditions (Adesakin *et al.*, 2020) of people using the well. This is further corroborated by the findings of Oyedele *et al.* (2019) who stated that the shallowness of the well makes it susceptible to unfitting disposal and penetration of effluent from solid waste, biological wastes, septic tanks and latrines into the groundwater. It is estimated that 842,000 deaths per year are as a result of inadequate water supply, sanitation and hygiene, mostly in low- and middle-income countries (Murray *et al.*, 2012).

Six bacteria species were identified in this study, which differs from the findings of Shahina *et al.* (2020) who reported 13 species of bacteria from both surface and underground water. The six bacteria species recovered in our study were also reported in their findings. Adesakin *et al.* (2020) did not detect any *S. aureus* in the borehole and tap water analyzed. However, they reported an abundance of *Proteus* spp. in tap and reservoir water than other bacteria while *Enterobacter* spp. were recovered mostly in river water. Agwaranze *et al.* (2017) reported *Staphylococcus aureus* as having the highest occurrence followed by *E. coli* (46.67%). *S. aureus* was not recovered from any of the water sources in the current study, meanwhile *E. coli* occurred more than any of the isolates. Shahina *et al.* (2020) reported that *E. coli, K. pneumoniae* and *E. aerogenes* occurred more in both underground and surface water than any of the other bacteria recovered. *Pseudomonas* was reported as the most prevalent in surface and drinking water in Mafikeng, South Africa (Mulamattathil *et al.*, 2014).

Many people living in rural areas lack proper hygiene and septic due to space limitations, crowding and a lack of a proper drainage network; thus the septic pit system is extensively used in this area, and seepage from these underground pits into the nearby wells might have contaminated the well water sources (Mukhopadhyay *et al.*, 2012). The presence of *E. coli* in water may be because of contamination from sewage or animal waste (EPA, 2001). It has been used as an indicator of water quality. WHO guidelines demand the absence of coliform of faecal origin in municipal drinking water supplies as reported by Javaid *et al.* (2022). According to the EPA, the presence of *E. coli* shows fresh sewage or animal waste contamination. Digging of pit latrines or septic tanks very close to boreholes and wells may result in the seepage of bacteria into these water sources. Also, the dumping of refuse inside water bodies can lead to an increase in bacteria load in the water which is corroborated by Lukubye and Andamaery's (2017) findings. *P. mirabilis* is predominantly a commensal of the gastrointestinal tract of humans and animals (Armbuster and Mobley, 2012) along with *E. coli* and *Klebsiella*, and causes serious infection.

The detection of moulds such as *Penicillium*, *Aspergillus* and *Trichoderma* is in line with the findings of Oliveria *et al.* (2016) and Ren *et al.* (2023) who reported the presence of these fungi in groundwater. Fungal pollution of water can lead to mycotoxin production, food contamination, odour and turbidity (Ren *et al.*, 2023). The presence of these fungi in water may be from the soil, crops, plant debris and organic matter (Hageskal *et al.*, 2009). Ayanbimpe *et al.* (2012) also reported the presence of *Candida* and *Rhodotorula* in water used for domestic purposes.

Shahina et al. (2020) reported that the Gram-negative bacteria recovered from water samples were 100% resistant to ceftazidime/clavulanic acid, amoxyclav followed by gentamicin, ceftriaxone and ceftazidime whereas they were susceptible to amikacin and ciprofloxacin. The isolates in this study also displayed a high resistance to ceftazidime and chloramphenicol. Mulamattathil et al. (2014) reported that coliforms from surface and drinking water are 100% susceptible to ciprofloxacin. Resistance to these antibiotics may be because of the production of enzymes capable of introducing chemical changes to the antibiotics (Wilson, 2014). Amarasiri et al. (2020) reported that the aquatic environment is one of the key routes for the spread of antimicrobial resistance. Pathogenic bacteria of human and animal origin that are released with wastewater into the water body harbour antibiotic-resistance genes and can spread among water and soil organisms (Alonso et al., 2001). In conclusion, this study has revealed the presence of pathogenic bacteria of human and animal origin. Some of these pathogens are responsible for water-borne diseases if taken directly and may likely cause food-borne diseases if this water is used in preparing foods without taking the necessary precautions. These bacteria are also resistant to some common antibiotics which called for concern. Also, the presence of toxin-producing fungi is of great health implications for humans and livestock. The government should endeavor to provide open lectures to the inhabitants of these rural dwellings on the possible aftermath of defecating in water bodies. Consumers of the water should also ensure they practice good hygiene in terms of boiling and filtering the water before drinking.

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