

BACTERIOLOGICAL ASSESSMENT OF DRINKING WATER FROM DIFFERENT SOURCES IN AGBOR, DELTA STATE

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Abstract

This study assessed the bacterial quality of drinking water from four commonly consumed sources in Agbor, Delta State, Nigeria: tap, well, bottled and sachet water. The assessment focused on bacterial load, presumptive identification of bacterial isolates, their distribution among the sampled water sources and antibiotic susceptibility patterns. Fifteen water samples were examined using Nutrient Agar, MacConkey Agar and Eosin Methylene Blue Agar, with colony morphology, Gram staining and biochemical and sugar-utilization tests used for presumptive identification. The colony counts varied substantially among individual samples. Well-water samples generally showed higher bacterial counts, and Sample 6 recorded the highest mean counts on Nutrient Agar (94 colonies), MacConkey Agar (34.3 colonies) and EMB Agar (14.7 colonies). Bottled-water samples generally showed lower counts, although Sample 11 recorded a relatively high Nutrient Agar mean of 85.3 colonies. Ten suspected bacterial isolates were recovered: *Corynebacterium* sp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Serratia* sp., *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. and two suspected *Escherichia coli* isolates. Both Gram-positive and Gram-negative organisms were detected, demonstrating considerable bacterial diversity. Antibiotic susceptibility testing showed variable responses, with resistance indices ranging from 0.2 to 0.7. *Staphylococcus aureus* had the lowest resistance index (0.2), while *Klebsiella* sp. and the second suspected *E. coli* isolate had the highest (0.7). Overall, the findings show that microbiological safety could not be assumed solely from water-source type. Regular microbiological monitoring, better protection of wells, improved hygiene during packaged-water production and handling, maintenance of tap-water distribution systems, and attention to antibiotic-resistant bacteria are recommended.

Keywords: Drinking water; bacterial contamination; well water; packaged water; *Escherichia coli*; antibiotic resistance

Introduction

Safe drinking water is essential to human health, and its microbiological quality is closely linked with the risk of waterborne disease. In Agbor, Delta State, residents obtain drinking water from a mixture of sources, including tap, well, bottled and sachet water. Differences in source characteristics, treatment, distribution and handling can influence the microbial quality of water available to consumers. The presence of organisms such as *Escherichia coli* is particularly important because it may indicate faecal contamination and the possible presence of other enteric pathogens. (Prüss-Ustün et al., 2019; World Health Organization, 2023)

Water can become contaminated at the source, during treatment, through distribution systems, during packaging or through subsequent transportation and storage. Groundwater from wells may be exposed to seepage, surface run-off, poorly protected well heads and nearby sanitary facilities. Tap water can be recontaminated through ageing or poorly maintained distribution systems. Although bottled and sachet waters undergo treatment or packaging, these processes do not automatically guarantee microbiological safety because contamination may occur through inadequate processing, poor sanitation, compromised packaging or unhygienic handling. (Ugbogu et al., 2021; Omoregie et al., 2021; Aneja, 2018)

The present study was undertaken to provide a localized assessment of drinking-water microbiology in Agbor by examining four commonly used water sources. The work determined bacterial colony counts, characterized recovered organisms using cultural, morphological and biochemical features, examined their distribution across sampled sources and evaluated their responses

to selected antibiotics. The study is important because localized evidence can help consumers, water producers, public-health personnel and relevant authorities identify potential risks and improve water-safety practices. (Tille, 2017; Janda & Abbott, 2007; Agi et al., 2022)

The study was guided by five practical objectives: to examine water samples from different sources for bacterial contamination; compare the microbiological quality of the different sources; assess their safety in relation to recognized water-quality expectations; provide information useful to residents of Agbor; and generate evidence that can support water-quality monitoring and management. (Omoriegie et al., 2021; Ugbogu et al., 2021)

Materials and Methods

The study examined 15 drinking-water samples collected from four source categories in Agbor, Delta State, comprising four tap-water samples, four well-water samples, four bottled-water samples and three sachet-water samples. The samples were subjected to conventional microbiological examination. Materials and apparatus included sterile sample bottles, conical flasks, test tubes, Petri dishes, cotton wool, aluminium foil, inoculating loops, sterile pipettes, measuring cylinders, glass slides, cover slips, a colony counter, laminar-flow cabinet, autoclave, incubator, microscope, analytical balance, forceps, filter paper, test-tube racks and a meter rule. The culture media included Nutrient Agar (NA), MacConkey Agar (MCA), Eosin Methylene Blue Agar (EMB), Mueller-Hinton Agar (MHA), urea medium and Simmons' citrate agar. Reagents included crystal violet, Gram's iodine, 95% ethanol, safranin, hydrogen peroxide, oxidase reagent, Kovac's reagent, bacterial plasma, bromocresol purple and the selected sugars, together with commercial antibiotic discs.

Nutrient Agar was prepared by dissolving 28 g of powder in 1 L of distilled water, while 55 g of MacConkey Agar and 36 g of EMB Agar were each dissolved in 1 L of distilled water. The media were sterilized by autoclaving at 121°C for 15 min, cooled to approximately 45–50°C and dispensed aseptically into sterile Petri dishes. For isolation, 1 mL of each water sample was transferred into 9 mL of sterile distilled water and allowed to stand for 30 min. Aliquots were plated on the prepared media and incubated at 37°C for 24 h. Resulting colonies were counted and recorded. The project calculated colony-forming units per millilitre using the expression: $\text{CFU/mL} = \text{number of colonies} \times \text{dilution factor} / \text{volume of sample plated (mL)}$.

Representative colonies were subcultured on Nutrient Agar to obtain pure cultures and subsequently maintained on Nutrient Agar slants. Cultural characteristics assessed included colony

size, shape, margin, elevation, consistency, colour and transparency. Gram staining was performed using crystal violet, Gram's iodine, 95% alcohol and safranin, followed by microscopic examination under the oil-immersion objective.

The presumptive identification process included catalase, urease, citrate-utilization, hydrogen-sulphide, oxidase, indole, coagulase and sugar-fermentation tests. Sugar fermentation was evaluated with lactose, sucrose, glucose, fructose, maltose, starch and sorbitol using peptone water and bromocresol purple as the indicator. For antibiotic susceptibility testing, Mueller-Hinton Agar was prepared using 38 g of powder per litre of distilled water and sterilized at 121°C for 15 min. The Kirby–Bauer disc-diffusion method was used with ten commercial antibiotic discs. Zones of inhibition were measured in millimetres after incubation at 37°C for 24 h, and the tests were performed in triplicate.

The equipment used in the study included a colony counter (Stuart SC6), laminar-flow cabinet (Esco Airstream AHC-4A2), autoclave (Hirayama HVE-50), incubator (Memmert IN30), microscope (Olympus CX23), and analytical balance (Ohaus Pioneer PX224).

Results and Discussion

Fifteen water samples were examined, with Samples 1–4 representing tap water, Samples 5–8 well water, Samples 9–12 bottled water and Samples 13–15 sachet water. Bacterial colony counts varied markedly among individual samples and across the three culture media. The results show that microbial quality differed from one sampling point to another rather than being determined exclusively by the broad source category.

Sample	Source	NA mean	MCA mean	EMB mean
1	Tap	45.0	17.7	9.7
2	Tap	41.3	22.0	5.0
3	Tap	78.3	29.0	8.0
4	Tap	76.7	19.0	12.3
5	Well	55.3	26.0	3.0
6	Well	94.0	34.3	14.7
7	Well	44.3	18.3	2.0
8	Well	33.7	9.3	2.0
9	Bottled	44.0	5.0	1.0

10	Bottled	54.0	16.7	2.0
11	Bottled	85.3	18.3	3.0
12	Bottled	27.0	10.0	3.0
13	Sachet	44.0	24.3	2.0
14	Sachet	36.0	15.0	4.7
15	Sachet	52.7	19.0	7.0

On Nutrient Agar, the mean counts ranged from 27.0 colonies in Sample 12 to 94.0 colonies in Sample 6. Sample 6, a well-water sample, also produced the highest mean counts on MacConkey Agar (34.3 colonies) and EMB Agar (14.7 colonies). The relatively high counts associated with some well-water samples are consistent with the vulnerability of groundwater where wells are poorly protected from surface run-off, seepage and nearby sanitary facilities. Tap-water samples also showed substantial bacterial growth, which may reflect contamination during distribution or inadequate protection of the supply system. Bottled water generally recorded lower counts, but Sample 11 was an important exception, with an 85.3-colony mean on Nutrient Agar. Sachet-water samples showed intermediate values, indicating that packaging alone did not eliminate microbial contamination.

The study therefore suggests a broad pattern in which well water was generally the most vulnerable source, bottled water was generally less contaminated, and tap and sachet water showed variable contamination. However, the individual sample variation is important: source labels alone were insufficient to predict microbiological quality. The relatively high count in one bottled-water sample demonstrates that contamination may occur during treatment, bottling, storage or distribution. (Omoregie et al., 2021; Ugbogu et al., 2021; Cabral, 2010)

Cultural and Presumptive Identification of Bacterial Isolates

Ten suspected bacterial isolates were recovered from the examined water samples. They comprised three Gram-positive organisms—*Corynebacterium* sp., *Bacillus cereus* and *Staphylococcus aureus*—and seven Gram-negative isolates comprising *Pseudomonas aeruginosa*, *Serratia* sp., *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. and two suspected *E. coli* isolates. Most colonies were cream-coloured and opaque, although the isolates differed in shape, size, elevation, margin and other cultural characteristics.

Suspected isolate	Gram reaction	Key reported reaction/feature	Primary medium
<i>Corynebacterium</i> sp.	Positive	Catalase positive; sucrose/glucose/fructose/maltose positive	NA
<i>Pseudomonas aeruginosa</i>	Negative	Oxidase positive; citrate positive	NA
<i>Bacillus cereus</i>	Positive	Catalase positive; starch positive	NA
<i>Staphylococcus aureus</i>	Positive	Catalase and coagulase positive	NA
<i>Serratia</i> sp.	Negative	Urease/citrate/sugar reactions supportive	MCA
<i>Klebsiella</i> sp.	Negative	Citrate and multiple sugar reactions supportive	MCA
<i>Enterobacter</i> sp.	Negative	Citrate positive; lactose/sugars positive	MCA
<i>Citrobacter</i> sp.	Negative	Citrate and H ₂ S reactions supportive	MCA
<i>Escherichia coli</i> 1	Negative	Indole positive; lactose positive; green EMB colony	EMB
<i>Escherichia coli</i> 2	Negative	Indole positive; lactose positive	EMB

The biochemical profiles supported the presumptive identities. The suspected *S. aureus* isolate was catalase- and coagulase-positive, while the suspected *P. aeruginosa* isolate was oxidase-positive. The two suspected *E. coli* isolates were indole-positive and lactose-fermenting, and one produced a green colony on EMB Agar. The occurrence of *Klebsiella*, *Enterobacter*, *Citrobacter* and *E. coli* is microbiologically important because these organisms can be associated with environmental or enteric contamination. Nevertheless, the project appropriately recognizes that cultural and biochemical tests provide presumptive rather than definitive species-level identification; molecular confirmation would be stronger for closely related organisms. (Ashbolt, 2004; Cabral, 2010)

Distribution of Isolates

The distribution pattern showed a single predominant suspected isolate at each of the ten points represented in the distribution table. *S. aureus* occurred at source 1; *Serratia* sp. at source 2; *Corynebacterium* sp. at source 3; *Klebsiella* sp. at source 4; *P. aeruginosa* at source 5; *Citrobacter* sp. at source 6; the first suspected *E. coli* at source 7; *B. cereus* at source 8; the second suspected *E. coli* at source 9; and *Enterobacter* sp. at source 10. This pattern indicates that contamination differed between collection points and may have been influenced by source-specific conditions such as hygiene, well protection, handling, packaging or storage.

Source point	Predominant suspected isolate
1	<i>Staphylococcus aureus</i>
2	<i>Serratia</i> sp.
3	<i>Corynebacterium</i> sp.
4	<i>Klebsiella</i> sp.
5	<i>Pseudomonas aeruginosa</i>
6	<i>Citrobacter</i> sp.
7	<i>Escherichia coli</i> 1
8	<i>Bacillus cereus</i>
9	<i>Escherichia coli</i> 2
10	<i>Enterobacter</i> sp.

Antibiotic Susceptibility Pattern

The recovered isolates showed variable susceptibility to the antibiotic discs tested. Resistance indices ranged from 0.2 to 0.7. Among the Gram-positive organisms, *Corynebacterium* sp. was sensitive to pefloxacin, levofloxacin and erythromycin; *Bacillus cereus* was sensitive to pefloxacin, ciprofloxacin, azithromycin, levofloxacin and erythromycin; and *S. aureus* was sensitive to pefloxacin and ciprofloxacin. Among Gram-negative organisms, *P. aeruginosa* remained susceptible to several agents, whereas *Klebsiella* sp. showed extensive resistance.

Isolate	Resistance index	Notable reported sensitivity	Notable resistance
<i>Corynebacterium</i> sp.	0.4	PEF, LEV, E	APX, Z, AM
<i>Bacillus cereus</i>	0.3	PEF, CPX, AZ, LEV, E	Z, AM, R
<i>Staphylococcus aureus</i>	0.2	PEF, CPX	Z, AM
<i>Pseudomonas aeruginosa</i>	0.2	AZ, LEV, CN, PEF, OFX	CPX, CF
<i>Serratia</i> sp.	0.3	AZ, LEV	AM, AU, CN
<i>Klebsiella</i> sp.	0.7	LEV	AM, AU, CN, PEF, OFX, CF
<i>Enterobacter</i> sp.	0.5	LEV, PEF	SP, CPX, AM, OFX
<i>Citrobacter</i> sp.	0.4	LEV, PEF	SP, AM, AU, OFX

<i>Escherichia coli</i> 1	0.5	PEF, AZ	LEV, AM, AU, OFX
<i>Escherichia coli</i> 2	0.7	LEV	AZ, SP, CPX, AM, AU, CN, OFX

Staphylococcus aureus recorded the lowest resistance index (0.2), while *Klebsiella* sp. and the second suspected *E. coli* isolate recorded the highest (0.7). Recovering organisms resistant to several antibiotics is relevant to drinking-water safety because water can serve as an environmental medium through which resistant bacteria may be disseminated (Ashbolt, 2004; Cabral, 2010). However, interpret the results using the appropriate organism- and antibiotic-specific susceptibility breakpoints, as inhibition-zone categories can vary by antimicrobial and organism. (Jorgensen & Ferraro, 2009; Berendonk et al., 2015)

Conclusion

The detection of suspected *E. coli*, *Klebsiella* sp., *Enterobacter* sp. and *Citrobacter* sp. indicate that some of the water sources may have been exposed to faecal or environmental contamination. The presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* also demonstrates the diversity of organisms that may enter drinking-water systems through environmental or human handling routes. Such contamination is particularly important for populations that may be more vulnerable to infection. The antibiotic resistance findings add another dimension to the public-health concern because water may contribute to the environmental circulation of resistant organisms.

The findings were broadly consistent with similar studies from other Nigerian locations. The project reports that packaged-water studies in Lagos have recovered organisms such as *Klebsiella*, *Enterobacter*, *Pseudomonas* and *E. coli*, while studies in Port Harcourt, Makurdi and Sokoto have also documented variable microbial quality and, in some cases, multidrug-resistant organisms. The similarity suggests that the observations in Agbor are part of a wider Nigerian concern in which water quality varies according to source protection, production, treatment, handling and storage. At the same time, the localized nature of the present assessment is valuable because conditions at individual water sources cannot be assumed to be identical across cities or communities.

The study demonstrates that the microbiological quality of drinking water in Agbor, Delta State, was not uniform across tap, well, bottled and sachet sources. Bacterial growth was detected in the examined samples, with considerable variation in colony counts. Some well-water samples showed particularly high bacterial counts, while bottled water generally recorded lower counts but was not

consistently free of contamination. Ten suspected bacterial isolates representing diverse Gram-positive and Gram-negative organisms were recovered, including organisms of public-health importance such as suspected *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas aeruginosa*. Antibiotic susceptibility results further showed that some isolates were resistant to multiple antibiotics, with resistance indices ranging from 0.2 to 0.7. Overall, the findings indicate that the microbiological safety of drinking water cannot be judged solely by whether it comes from a tap, well, bottle or sachet; treatment, sanitary protection, handling, distribution and storage all require attention. Therefore, regular microbiological monitoring and improved sanitary practices are essential to ensure the safety of drinking water and reduce the potential public health risks associated with microbial contamination and antibiotic-resistant bacteria (Prüss-Ustün et al., 2019; World Health Organization, 2023).

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