**Physicochemical and microbiological characteristics of surface and groundwater frequently used by households in some districts of Daloa (Côte d'Ivoire)**

**Abstract**

Due to water supply shortages in Daloa, residents are turning to different sources, such as surface water and groundwater, for their needs. However, the quality of these waters is not monitored, posing serious health risks. This study aims to assess the physicochemical and microbiological characteristics of the various water sources used in the city.

The methodology involved sampling from two groundwater sites, with 60 samples taken (20 for each type of water, 10 from each site). Physicochemical analyses included pH, temperature, and heavy metals (Fe, As, Cd, Hg, Pb). Microbiological analysis focused on Aerobic Mesophilic Germs (AMG), Fecal and Total Coliforms, fecal streptococci, and fungal flora. After identifying specific strains, an antibiogram was conducted.

Results show that borehole and well water meet WHO standards while surface water is highly polluted. Microbial loads are as follows: GAM (405.105-659.105 CFU/mL), (685.103-766.103 CFU/mL), Fecal Streptococci (39.102-45,102 CFU/mL), and fungal flora (659. 103-685,103 CFU/mL) exceeding WHO standards. Microbial strains identified include *Listeria sp*, *Citrobacter diversus*, *E. coli*, *Enterobacter aerogenes*, *Pseudomonas sp*, and *Klebsiella oxytoca* in lake and well water. The identified strains are multidrug-resistant but sensitive to colistin, an antibiotic of last resort.

**Keywords:** Groundwater, surface water, heavy metals, multidrug-resistant, Daloa

**Introduction**

Waterborne diseases have increased worldwide, particularly in developing countries (Ako et al., 2009; Jadhav, 2011; Nyamai et al., 2021). These diseases primarily affect children, the elderly, and immunocompromised individuals (Freeman et al., 2012). The World Health Organization reports that approximately 600,000 children die from diarrheal diseases caused by a lack of clean water, sanitation, and hygiene. Contaminated water can lead to bacterial infections, epidemics (Breitenmoser et al., 2011), severe health issues, and significant social and economic losses (Emmanuel et al., 2009). Diarrhea is the most prevalent illness associated with consuming water contaminated by pathogens through the fecal-oral route.

One of the Millennium Development Goals' primary objectives was to reduce the population without access to clean water to 50% by 2015 (Rubino et al., 2019). Unfortunately, this target has not been met, and to date, over 30% of the world's population still lacks access to domestic drinking water services. The challenging access to water is particularly acute in developing countries, where the quantity and quality of water resources are at risk (Ahoussi et al., 2013). Access to clean water is crucial for improving living conditions, as the lack of water encourages water consumption from various sources, posing health risks (Kouadio et al., 2020).

In sub-Saharan Africa, poor water quality leads to over half a million deaths from diarrheal diseases annually. Bacteria associated with poor water quality include *Enterobacteriaceae*, *Entamoeba histolytica*, and *Aeromonas* sp (Maran et al., 2016; Nyamai et al., 2021). In Côte d'Ivoire, access to water remains a major concern for the population (Yapo et al., 2016; Kanohin et al., 2017; Ahoussi et al., 2013). Despite various projects to provide water to all localities, challenges persist, and drinking water is still inaccessible (Mangoua et al., 2010). Many towns have inadequate drinking water coverage, compounded by urbanization, deterioration of water supply infrastructures, and insufficient financial resources for rehabilitation and expansion of facilities (N'Guessan, 2009). This results in poor water quality in some areas, including Daloa in south-west Côte d'Ivoire.

In Daloa, only 4.3% of households in vulnerable neighborhoods are connected to the water management and distribution company, while 95.7% are not (Awomon et al., 2019). Consequently, residents resort to alternative water sources such as natural springs, borehole water, traditional wells, and bagged water, which are not without health risks and may lead to waterborne diseases due to bacteria and heavy metals such as Arsenic (As), Cadmium (Cd), and Mercury (Hg) (Charlet and He, 2013; Affum et al., 2015).

This study aims to assess the physicochemical and microbiological quality of certain surface and ground waters used domestically in Daloa, which may pose health concerns due to their extensive use. Specifically, we are investigating the presence of heavy metals such as Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb), as well as certain microorganisms, including Mesophilic Aerobic Germs (MAG), *Escherichia coli*, fecal and total coliforms, fecal streptococci, and fungal flora, while also determining their antibiotic resistance profile.

**Materials and methods**

**Description of the study area**

This study occurred in Daloa, a town in the western part of Cote d'Ivoire. Daloa's GPS coordinates are longitudes 6°24' to 6°29' West and latitudes 6°50' to 6°55' North. Daloa is also known as the capital of the Haut Sassandra region. It is 141 km from Yamoussoukro, the political capital, and 383 km from Abidjan, the economic capital of Cote d'Ivoire. The town covers an area of 17,761 km2 and has an estimated population of 705,378 (RGPH, 2022).

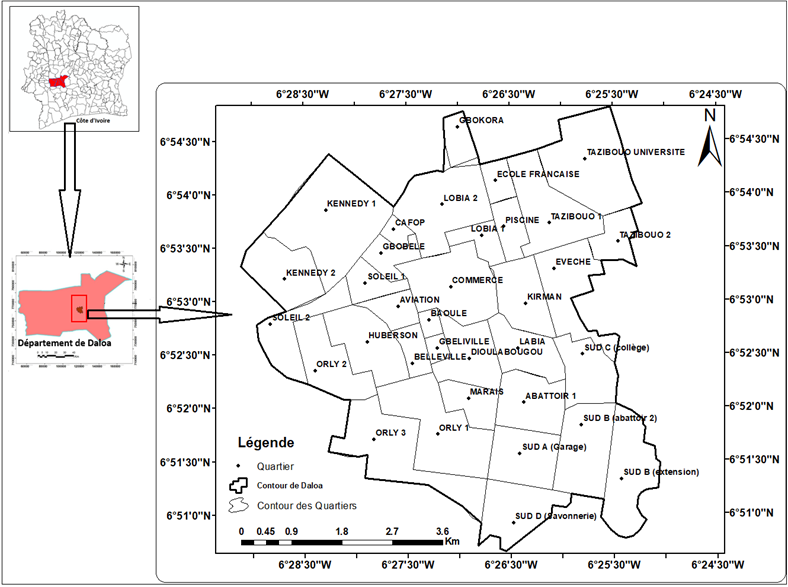


Figure 1: Map of the city of Daloa and its districts (Kouassi, 2021)

**Sample**

The water sources used in this study are from 5 districts of Daloa: Lobia, Tazibouo, Olivier, Gbokora, and Commerce (Figure 1). These districts have primary water supplies used for various human activities. Water samples were collected in the 5 identified districts between March 10 and April 25, 2023.

Groundwater samples were taken from wells and boreholes, and well water samples were taken in the Tazibouo and Oliviers districts. Borehole water samples were taken in the Tazibouo district, at the Diocesan Reception Center (CAD), and at the Lobia water tower. For surface water, samples were taken from lakes in the Commerce and Gbokora districts. One sampling site per type of water and district was used. The various water samples were taken in sterile 500 mL bottles, which were then coded. A total of 60 samples were collected, with 20 for each type of water (10 for each site). The samples were transported to the laboratory in 3 different refrigerated coolers (one for every kind of water: well water, spring water, and lake water) and analyzed as soon as they arrived.

**Physicochemical Analyses**

**Temperature and pH**

The temperature of water samples is measured on-site at each water sampling location. We used an FY-10 Thermometer from China with an electronic display, which was turned on a few minutes before taking any measurements. For spring and well water samples, the thermometer probe is placed in the water collected in a 50 mL Falcon tube. When sampling lake water, the probe is placed directly in several points of the lake, and the recorded value is the average of the readings from these points.

The pH of the different water samples is measured in the laboratory using a pH meter from Hanna, Romania. The pH meter probe is placed directly into 50 mL of water in a 100 mL beaker.

**Metallic trace elements**

Traces of heavy metals (Fe, As, Cd, Hg, Pb) in the samples were determined by inductively coupled plasma optical emission spectroscopy (ICP/OES) according to the protocol of Bozorgzadeh et al. **(2021)**.

**Microbiological Analyses**

The samples were subjected to microbiological analysis using a series of dilutions. Mother suspensions and decimal dilutions were prepared following the NF EN ISO 6887-1 recommendations. The microbial analysis was conducted following the standards outlined in Table 1.

**Table 1: Water quality assessment criteria**

|  |  |  |
| --- | --- | --- |
| **Researched germs** | **Criteria** | **References** |
| **Fungal flora** | 105 UFC/100 mL | 76/160/EEC of 8 December 1975 Decree 91-980 of 20 September 1991 |
| ***E. coli*** | Absence/100 mL |
| **Fecal coliform** | 10 UFC /100 mL |
| **Fecal Streptococci** | Absence/100 mL |
| **Coliform totals** | 10 UFC /100 mL |
| **Enterobacteria** | 10UFC/100 mL |
| **Mesophilic Aerobic Germs** | 105 UFC/100 mL |

**Research for Mesophilic Aerobic Germs (MAG) (Standard NF/ISO 4833:2003)**

We used Plate Count Agar (PCA) to enumerate the GAM according to the standard procedure. We took 1 mL of each dilution using a sterile pipette and placed it in a Petri dish. Then, we poured approximately 20 mL of the PCA medium, previously prepared and kept cool, into the Petri dish. The contents were homogenized manually using light circular movements for 1 minute. After solidification, we placed the dishes in an incubator at 30°C for 48 to 72 hours, and only plates with colonies between 30 and 300 were used for counting.

**Total coliform and fecal coliform tests (Standard** **ISO 4832: 2006 and ISO 21528-2: 2004)**

VRBL culture medium (Biokar, France), previously prepared and maintained in supercooling at 45°C, is inoculated with 1 mL of each dilution. The plates are then placed in an oven at 44°C for fecal coliforms and 37°C for total coliforms. After 24 h incubation, the characteristic colonies are purplish, 0.5 mm or more in diameter, sometimes surrounded by a reddish zone.

**Fecal streptococci Research (standard ISO7899-2:2000)**

The search for fecal streptococci was carried out on BEA agar (Biokar, France), previously prepared and poured into Petri dishes (to a quantity of 20 mL). They were inoculated at the surface with 0.1 mL of the chosen dilution. The culture was incubated at 37°C for 24 h. Colonies present on the different black plates are counted.

**Fungal flora search**

To test for fungal diversity in samples of different types of water, Sabouraud chloramphenicol agar (Alpha Biosciences, USA), previously prepared and maintained in supercooling, was inoculated with 1 ml of each dilution prepared. Incubation took place at 30°C for 3 to 7 days. Mold colonies are pigmented, velvety, cottony, and more or less swollen, while yeast colonies resemble those of bacteria. They may have regular or irregular edges, convex or flat shapes, and are often opaque.

Conventionally, the identification of fungal genera is based on morphological criteria: on the one hand, macroscopic observation of the mycelium, and on the other, microscopic observation of reproductive structures.

Macroscopic observation: observation with the naked eye to determine morphological and cultural characteristics (growth rate, appearance of mycelium, color of mold colonies after inoculation of pure strains on specific culture media). Microscopic observation: observation of hyphal compartmentalization, presence of chlamydospores, shape, and size of conidia, phialides, etc.) from a sampled mycelium fragment **(Domsch et al., 1980)**.

**Biochemical tests to identify bacterial strains**

Characteristic colonies from specific media are re-isolated on PCA media. After Gram staining, two to three colonies are transferred to Leminor's reduced staining media.

**Antibiogram test of identified isolates**

Pure cultures of the species obtained were used to prepare the inocula. To this end, a suspension was prepared in a hemolysis tube by bubbling pure 24 to 48 h colonies obtained on nutrient agar into 2 ml of sterile physiological water. Once thoroughly homogenized, the suspension is read on the Densimat and adjusted to 0.5 McFarland opacity. The final inoculum of the species is obtained by adding 100 µl of this suspension to 10 mL of sterile physiological water in a screw-top test tube. This final suspension, containing around 108 CFU/mL, is the inoculum used for the antibiogram.

Mueller Hinton agar (BioRad, France) was used for antibiotic susceptibility testing. It was prepared according to the manufacturer's recommendations. The prepared medium was poured into Petri dishes at a rate of 20 mL per Petri dish. 3 mL of pre-prepared inoculum is poured onto the surface of Mueller Hinton agar. After a contact time of 5 minutes, the excess inoculum is removed with a pipette. Using a sterile swab, excess inoculum is removed by pressing on the edges of the plate at a 60° angle. The dish is dried at 37°C for 3 min. Antibiotic discs (BioMérieux, France) were aseptically applied to the agar surface using forceps.

The antimicrobials tested were Kanamycin (KAN), Aztreonam (AZT), Imipenem (IPM), Piperacillin (PIP); Cefuroxin (CXM); Cefepim (CEF), amikacin (AMK); ampicillin (AMP); Ceftazidime (CAZ); Ciprofloxacin (CIP), colistin (CL); gentamicin (GM). Depending on the inhibition diameter, the strain is classified as sensitive (S), intermediate (I), or resistant (R) to the antibiotic, group, or families of antibiotics according to the manufacturer's prescriptions (CA-SFM, 2020). According to Maran et al. (2016), Bacteria resistant to two or more antimicrobials were classified as multidrug-resistant.

**Results and Discussion**

**Results**

**Temperatures and pH Values of the various water sources**

The table below shows the temperature and pH values of the various water sources studied. Lake water is very high, at 31°C, compared with an average of 24°C for borehole and well water. Lake water is also highly acidic, with pH values (4.10-4.30) below those recommended by the WHO (6.5-8.5).

**Table 2: Temperature and pH of the various water sources**

|  |  |  |  |
| --- | --- | --- | --- |
| Types of water | | Means temperatures | Means pH |
| Drilled wells water | **from lobia** | 24.10 ±0.78 | 6.30 ±0.56 |
| **from CAD** | 23.90 ±1.02 | 6.27 ±0.42 |
|  |  |  |  |
| Well water | **from Tazibouo** | 24.3 ±0.75 | 6.91 ±0.52 |
| **from Olivier** | 25.1 ±0.45 | 6.83 ±0.74 |
|  |  |  |  |
| Lake water | **from commerce** | 31.12 ±0.36 | 4.10±0.68 |
|  | **from Gbokora** | 31.59 ±0.25 | 4.30±0.41 |
|  |  |  |  |
| Normes OMS 2017 | | **\*24.2°C** | **6.5–8.5** |

**Heavy metals in the various water sources**

Research into heavy metals in the different types of water shows a variation from one kind of water to another. Levels remain below WHO standards for mercury, Arsenic, and Cadmium. On the other hand, iron levels in lake water remain very high, ranging from 2023 µg/L to 3031 µg/L for Commerce and Gbokora lakes, respectively. Lead levels in groundwater remained below the norm, reaching 8.31 and 7.91 µg/L for spring water and 6.99 and 7.59 µg/L for healthy water.

**Table 3: Heavy metal content in different types of water**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Types of water | | Heavy metals (µg/L) | | | | |
| **Mercury (Hg)** | **Arsenic (As)** | **Cadmiun (Cd)** | **Plomb (Pb)** | **Fer (Fe)** |
| Drilled wells water | **from Lobia** | 0 | 1.03 | 0.01 | 8.31 | 998.65 |
| **from CAD** | 0 | 1.20 | 0 | 7.98 | 1032 |
|  |  |  |  |  |  |  |
| Well water | **from Tazibouo** | 0 | 1.8 | 00 | 7.59 | 765 |
| **from Olivier** | 0 | 1.6 | 00 | 6.99 | 743 |
|  |  |  |  |  |  |  |
| lake water | **from Commerce** | 0.41 | 2.02 | 0.3 | 13.14 | 2023 |
|  | **from Gbokora** | 0.96 | 2.7 | 0.6 | 17.36 | 3031 |
|  |  |  |  |  |  |  |
| Standard of OMS 2017 | | **5** | **10** | **3** | **10** | **300** |

**Microbiological quality of studied waters**

Microbiological analysis assessed the microbial quality of the various water sources studied. The following germs were tested: MAG, fungal flora (yeasts and molds), fecal streptococci, fecal coliforms, and E. coli. The various results obtained are presented in Table 4. The GAM load is lower in borehole water, between 145 and 201 CFU/100 mL. These values are below the WHO recommended standard of 105 CFU/100 mL. There was an absence of fecal coliforms in these waters.

**Table 4: Microbial load of the different types of water under investigation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Type of water | | Microbial loads (UFC/100mL) | | | |
|  | | **GAM** | **LM** | **CF** | **SF** |
| Drilled wells water | **from Lobia** | 145 ± 98a | 30±10 a | 0±00 a | 3±30 a |
| **from CAD** | 201 ±31 b | 10±03 a | 00±00 a | 7±10 a |
|  |  |  |  |  |  |
| Wells water | **from Tazibouo** | 129.103 ±208 a | 1575± 83a | 52.102± 484,29b | 63±12c |
| **from Olivier** | 127.103 ±324ab | 1231± 47b | 48.102± 388,90 | 52 ±23c |
|  |  |  |  |  |  |
| Lake water | **From Commerce** | 405.105 ±1010c | 685.103±502a | 371.104 ±2041c | 39.102± 749d |
|  | **from Gbokora** | 659.106 ±1322 d | 766.103±203b | 432.104 ± 1606c | 45.102± 147a |
|  |  |  |  |  |  |
| OMS Standard 2017 | | **105 ufc/100mL** | **105 ufc/100mL** | **Abs/100mL** | **10 ufc/100mL** |

NB: Values assigned the same letter are statistically identical, at the 5% threshold

**Microbial diversity of the water analyzed**

**Bacterial species**

Table 5 lists the different bacterial species found in the waters analyzed. Lake water showed the greatest bacterial diversity: *Listeria sp* (01), *Citrobacter diversus* (01), *E. coli* (02), *Enterobacter aerogenes* (03), *Pseudomonas sp* (04), *Klebsiella oxytoca* (01)), followed by well water (*Enterobacter aerogenes,* *Klebsiella oxytoca*) and drilled well water (none).

**Table 5: Distribution of bacterial species in different types of water**

|  |  |
| --- | --- |
| **Types of water** | **Bacterial species found** |
| Lake water | *Citrobacter diversus* (1)*, E. coli sp*, *Enterobacter aerogenes sp* (3), *Pseudomonas sp* (4), *Listeria sp* (1) |
| Well water | *Enterobacter aerogenes sp*  (1), *Klebsiella oxytoca sp* (1) |
| Drilled well water | None |

**Distribution of fungal species in different types of water sources**

Table 6 lists the fungal species found in the waters analyzed. Fungal diversity varies from one type of water to another. Common contaminant species are the genera *Aspergillus sp* and *Trichoderma sp*.

**Table 6: Distribution of fungal species in different types of waters**

|  |  |
| --- | --- |
| **Types of water** | **Fungal species found** |
| Lakes water | - *Aspergillus nidulans (01) ; Trichoderma sp. (02) ; Trichoderma harzianum (01) ; Beauveria sp. (01) ; Scopulariopsis brevicaulis(01) ; Onychochola sp. (03); Aspergillus fumigatus (02) ; Aspergillus niger (02) ;Aspergillus versicolor (01).* |
| Wells water | *Trichoderma sp. (02) ; Trichoderma harzianum (01) ; Beauveria sp. (01) ; Scopulariopsis brevicaulis(01) ; Onychochola sp. (03); Aspergillus fumigatus (02) ; Aspergillus niger (02) ;Aspergillus versicolor (01).* |
| Drilled wells water | *Trichoderma, Fusarium, Penicillium et Cladosporium* |

**Antibiotic resistance of bacterial strains**

Antibiotic susceptibility testing was conducted on the strains to determine their resistance to the various antibiotics tested. The results are shown in Table 6 below. Most strains were resistant to at least 2 antibiotics. *Citrobacter diversus* strain resists more than half the antibiotics tested (7/12). All strains are sensitive to colistin.

**Table 6: Antibiotic resistance of isolated strains**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strains | Antibiotics tested | | | | | | | | | | | |
|  | **KAN** | **AZT** | **IPM** | **PIP** | **CXM** | **CEF** | **AMK** | **AMP** | **CAZ** | **CIP** | **CL** | **GM** |
| *Pseudomonas* | R | R | S | R | S | S | S | R | S | S | S | S |
| *Enterobacter aerogenes* | R | R | S | S | R | R | S | R | S | S | S | S |
| *klebsiella oxytoca* | R | R | R | R | R | R | S | I | S | S | S | S |
| *citrobacter diversus* | R | R | R | R | R | R | S | R | S | S | S | S |
| *E. coli* | S | R | S | R | R | R | R | S | S | S | S | S |

**Kanamycin (KAN), Aztreonam (AZT), Imipenem (IPM), Piperacillin (PIP), Cefuroxin (CXM), Cefepim (CEF), amikacin (AMK), ampicillin (AMP); Ceftazidime (CAZ); Ciprofloxacin (CIP), colistin (CL); gentamicin (GM).**

**Discussion**

Accessing drinking water is difficult in various regions of Côte d'Ivoire, especially in Daloa, forcing people to use available groundwater and surface water (Awomon et al., 2019). Our study examined the physical, chemical, and microbiological quality of water sources in the Commerce, Tazibouo, Gbokora, and Oliviers districts. The sources of supply include wells, natural lakes, and drilled well water.

Lake water has an acidic pH (4.10 and 4.30) and high temperatures above 30°C, likely due to daily exposure to solar radiation. The average annual temperature in Daloa is around 30°C (Yao et al., 2015). The low pH is below WHO standards, attributed to human activities such as domestic wastewater and household waste discharges into the lakes. Furthermore, these activities produce CO2 from the decomposition of organic compounds, leading to the acidity of the water (Ahoussi et al., 2013). Well and drilled well water mostly meets WHO standards (24.2°C and pH 6.5-8.5) due to less exposure to sunlight and environmental pollution. The observed low pH in these samples may result from the acidic Latossol soil of these regions (Maran et al., 2016).

Iron levels in different water types exceed the WHO standard (≤ 300 µg/L), with spring water having iron levels of 998.65 and 1032 µg/L at the Lobia and CAD sites, respectively. Surface waters have even higher levels, around 10 times the recommended standard. This is particularly evident in Gbokora Lake, with an iron content of 3,000 µg/L. These findings are likely due to the region's geology, mainly comprised of ferromagnesian rocks, which can lead to the accumulation of iron in soils and subsequent leaching into groundwater (Naho, 1988; Djadé et al., 2020).

Regarding microbiological analysis, groundwater is less contaminated than surface water in our study. Spring water has the slightest contamination, with no fecal coliforms present. This disparity may be due to groundwater being protected from airborne pollution, unlike surface water. However, some wells are regularly treated with chlorine, which inhibits pathogenic microorganisms' proliferation (Belghiti et al., 2013). Bacterial strains, mainly from the *Enterobacteriaceae* family, are more prevalent in surface waters, likely originating from run-off water. Groundwater showed an absence of *E. coli*. Similarly, fungal flora, including Aspergillus sp and Trichoderma sp, is present in different types of water in the study, with surface waters being the most contaminated. Their presence in groundwater is attributed to their ability to form biofilms due to humidity (Maran et al., 2016).

The antibiograms conducted on the different identified strains show a resistance profile to more than two antibiotics, indicating that they are multidrug-resistant bacteria (Maran et al., 2016). Previous research studies in the Daloa region, including those by Kouassi et al. 2020 and Zébré et al. (2022), have also observed multidrug resistance in environmental strains. The Citrobacter strain exhibits the highest multi-resistance, with 58% resistance.

This finding suggests that resistant bacteria in water indicate the presence of pathogenic organisms that can spread infection among groundwater consumers (Mukhopadhyay et al., 2012). On the other hand, all the strains are sensitive to colistin, one of the last-resort antibiotics (Lim et al., 2010). The emergence of resistance mechanisms in certain bacteria to this last-resort antibiotic remains a public health problem, as it is generally used for patients with complex infections (Falagas et Kasiakou, 2005; Doret et al., 2016).

Upon analyzing these multi-resistant strains, most were found in surface waters, particularly lakes. These waters are often affected by human activities. Human activity can spread resistant micro-organisms, potentially resulting in the dissemination of multidrug-resistant agents in hospitals, communities, and the environment, posing a public health problem (Wellington et al., 2013; Larsson et Flach, 2022).

**Conclusion**

The study provided important information on the quality of surface and groundwater extensively used in the Lobia, Tazibouo, Olivier, Commerce, and Gbokora districts of Daloa. The results showed that the borehole water meets the recommended WHO standards. Surface water is the most contaminated, with various types of microbiological contamination, including bacteria and fungi. The bacteria found in surface water, particularly in lakes, are multi-resistant and sensitive to colistin, an antibiotic of last resort.

Surface water is more contaminated than groundwater. Most households surveyed in Daloa use spring and well water for drinking and cooking, while lake water is used for activities like swimming, agriculture, and construction. Well water and lake water contain AMG, fecal and total coliforms, *E. coli*, and fungi flora. These germ levels are higher in surface water (lake) compared to the standard, indicating poor water quality. On the other hand, borehole water does not contain any microorganisms and is of satisfactory quality, making it suitable for human consumption.

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