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ANTIBIOTIC SENSITIVITY/RESISTANCE OF BACTERIA ISOLATED FROM GROUNDWATER AT THE SOURCE AND UNDER HOUSEHOLD STORAGE CONDITIONS IN YAOUNDÉ (CAMEROON, CENTRAL AFRICA).

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ABSTRACT

A study was conducted on groundwater sources and household storage conditions in selected homes in the city of Yaoundé (Cameroon) from January to March 2025. Water samples for bacteriological analysis were collected using standard methods. The bacteriological analyses considered the total viable flora and pathogenic bacteria. After identifying the microorganisms using the classical gallery method, antibiograms were performed according to the recommendations of the Antibiogram Committee of the French Society for Microbiology and the European Committee on Antimicrobial Susceptibility Testing, based on antibiotics commonly used in the city. Moreover, in addition to the MAHB, the waters sampled in the households and in the water, supply sources harbor pathogenic bacteria such as Serratia marcescens, Citrobacter freundii, and Salmonella paratyphi. The densities of these bacteria undergo overall variations depending on the time of water collection, the household, and the water source considered. In addition, the physicochemical parameters considered were taken in situ and in the laboratory using standard techniques. The microbial group under investigation exhibited resistance to amoxicillin-clavulanic acid, ceftazidime, cefotaxime, and cefepime. This result suggests that the contamination may be due to storage conditions. It is therefore recommended that populations use cleaner and better (watertight and non-porous) containers and continue to treat the water regularly before use.

Keywords: antibiotic, sensibility/resistance, water, storage condition

INTRODUCTION

The water consumed every day is essential to life (servais *et al*., 2007). Its quality has always been an indispensable element of an environment conducive to health. Currently, far from having been resolved, the problem of the quality of drinking water is still a public health priority, both for emerging and industrialized countries (servais *et al*., 2007). In emerging countries, the scourge of water-borne enteric diseases is still just as glaring a problem and it is a pity to note that the United Nations' sustainable development objectives aimed at "access for all to drinking water" have never been reached and are no longer even targeted by this body (WHO, 2004).

Access to quality drinking water is a fundamental right for all populations and a major public health issue. According to the World Health Organization (WHO, 2022), approximately 2 billion people worldwide consume drinking water contaminated with fecal matter, causing more than 485,000 deaths annually due to diarrheal diseases (WHO, 2022). In sub-Saharan Africa, reliance on groundwater sources is high, particularly in urban and peri-urban areas where water distribution networks are often inadequate or nonexistent.

In large African cities like Yaoundé, the political capital of Cameroon, rapid population growth combined with uncontrolled urbanization has placed significant pressure on water resources (Njine *et al*., 2001). Given the insufficient coverage of the CAMWATER network, many households resort to alternative sources such as wells and boreholes that exploit groundwater. These waters are generally perceived as pure since they are protected from surface pollution (Nola *et al*., 2006, 2010; Noah Ewoti, 2012). However, several studies have shown that these resources are not immune to contamination, particularly of bacterial and chemical origin (Tabué Youmbi *et al*., 2013; Moussima Yaka *et al*., 2020, Noah Ewoti *et al*, 2021b).

While the soil absorbs and filters many contaminants, smaller particles such as microorganisms can be transported through cracks in rock or permeable soils, and then reach the aquifer (Nola *et al*., 2000; 2010). Thus, thin soil layers and high-water tables contribute to groundwater vulnerability (Nola *et al*., 2010; Noah Ewoti *et al*., 2021d). Both bacteria and pathogenic parasites originate from animal and human faeces (Holt *et al*., 2000). Until the 20th century, the potential effects of groundwater consumption on health were little known, especially since it was deemed to be safer than surface water. In Cameroon, recent studies have shown the gradual and continuous degradation of the microbiological and physicochemical quality of watercourses (Ntsama *et al*., 2017; Noah Ewoti *et al*., 2021a). These studies have shown that in the city of Yaoundé and its surroundings, surface watercourses can harbor a pathogenic microflora consisting of enterobacteria enteropathogens and enterotoxigens, several species of vibrios and pseudomonaceae (Noah Ewoti *et al*., 2021b ; Tamsa *et al*., 2021). Faced with these germs, the most appropriate antibiotics are

Ciprofloxaxin (32.5%) and sulfamethoxazole / trimethoprim (35%) while the most resistant are ampicillin (57.5%), followed by ofloxacin (55%), amoxicillin (50%) (Eheth *et al*., 2016; Mafany *et al*., 2021). These authors indicate that the resurgence of waterborne diseases caused by surface waters have led populations to adopt groundwater for their food because of their apparent clarity but in ignorance of their microbiological quality (Zebaze Togouet *et al*., 2011; Moungang *et al*., 2013).

This last piece of information poses the prelude to the problems of updating data on the accommodation of pathogenic bacteria by groundwater in general and those offered by the Decentralized Territorial Communities (CTD) to populations in particular in emerging countries. In addition, very few studies elucidate the presence of bacteria in borehole and surface water used for drinking water and sensitivity of these germs to antibiotics from bacteria isolated from underground water in source and storage condition in household.

The present work aims to evaluate the antibiotic sensitivity of bacteria from underground water points in source and storage water in household in Yaounde (Centre Cameroon).

MATERIAL AND METHODS

Study framework

The selection of sampling points makes it possible to obtain an overall picture of the water quality throughout the distribution chain, which allows for monitoring the evolution of the bacteriological quality of the water throughout the process. It is important to apply good sampling practices, as sampling is one of the most critical steps in the evaluation of water quality. Therefore, sampling must be carried out carefully to avoid all possible sources of contamination. To ensure this: The study sites were chosen based on their accessibility, the presence of pollution sources, and the interest of local populations in these water sources. Using these criteria, four sampling points were selected: stations F1 (Borehole 1), F2 (Borehole 2), S1 (Spring 1), and S2 (Spring 2), all located in the Mendong neighborhood in the Yaoundé VI district. This neighborhood has a very high population density, estimated at 200,000 inhabitants per square kilometer. The various sampling sites are shown in Figure 1.

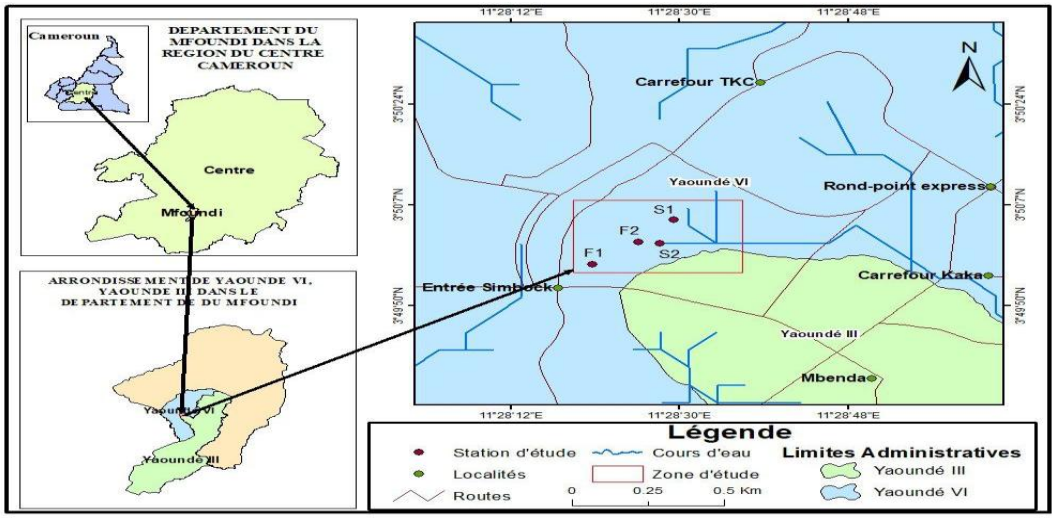


Figure 1: Geographical Positions of Sampling Points in the Yaoundé 6 District, Mfoundi Department, in the Centre Region

Description of Sampling Points

Figure 2 (F1; S1) shows the water sources analyzed during the study period, characterized by the presence of a specific point source of pollution. Overall, these points are located between 3°49'59.61'' and 3°50'1.12452'' North latitude and between 11°28'27.66'' and 11°28'29.39304'' East longitude. For household monitoring, water was stored in the first household in a barrel (figure 2A) whose lid was regularly opened for water usage. This water was used for drinking, cooking, dishwashing, and bathing. In this first household, the water supply came from borehole F1. In the second household, water was stored in cleaned 10-liter jugs (figure 2B). This water was used for serving drinks to customers of a small food business and for the family's drinking needs. The source of supply is station S1.

Figure 2: Photograph of the Sampling Points During the Study Period (F1, S1), and Water storage containers in use in households A and B (Photo by Npah Ewoti, February 2025)

Sampling Methods

Samples were collected using two types of containers: polyethylene bottles of 250 mL and 1000 mL, which were thoroughly washed and rinsed in advance at the laboratory, and sterile glass bottles of 500 mL. The water was sampled according to the techniques recommended by APHA (2009) and Rodier *et al.* (2009). For each sample, two types of parameters were considered: physicochemical and microbiological (Rodier *et al*., 2009).

These samples were then transported to the laboratory in a refrigerated chamber, and the analyses were conducted within hours of sampling. The water samples contained in polyethylene bottles were used for physicochemical analyses, while those in sterile glass bottles were used for bacteriological analyses.

Bacteriological Analysis

Isolation and Enumeration of Mesophilic Aerobic Heterotrophic Bacteria (MAHB)

MAHB species were isolated on the surface of PCA (Plate Count Agar) medium poured into Petri dishes using the surface spread technique. To do this, 100 µl of raw sample were taken with a sterile micropipette and deposited onto the agar surface. The sample was then spread using a sterile glass spreader within the sterile flame zone of a Bunsen burner (Marshal *et al*., 1991). The Petri dishes were then incubated at room temperature for 5 days. During this period, colonies with diverse cultural characteristics were counted using the direct counting method (Holt *et al*., 2000).

Isolation of Pathogens

Isolation was done by taking 100 µl of the raw sample using a micropipette. Near the flame of a gas-fed Bunsen burner, this volume was spread across the surface of MacConkey agar and Salmonella-Shigella (SS) agar media poured into Petri dishes. The plates were incubated at 37°C in an incubator for 24 to 36 hours (Delarras, 2007).

Enumeration of Viable Pathogens

The colonies isolated and showing the cultural characteristics of suspected strains were counted using the direct counting method. Bacterial abundances were first expressed as Colony Forming Units (CFU)/100 mL of water sample examined, then transformed into log CFU/100 mL to better represent the variation and reduce high differences in densities of the targeted bacteria (Holt *et al*., 2000; Noah Ewoti *et al*., 2011; Moungang *et al*., 2013).

Identification of Pathogens

After isolating the pathogens and macroscopically recognizing the colonies, the identification of different bacterial genera was conducted using microscopic descriptions of the cells based on their relevance in the medical field. Biochemical or enzymatic tests were performed after culturing pure strains on ordinary slanted agar media in test tubes.

Biochemical Identification of Pathogens

The identification of various bacterial species was based on a series of biochemical tests. After subculturing on slanted PCA medium and incubating at 37°C for 18 to 24 hours, the biochemical tests were carried out according to standard biochemical criteria, using the classic Le Minor gallery (Holt *et al*., 2000). This gallery includes a set of reactions whose results correspond to the phenotype and reveal the genetic configuration specific to the tax on being studied.

Antibiotic Susceptibility Profile (Antibiogram)

Sensitivity Profile of Isolated Strains

Antibiotic susceptibility testing (antibiogram) was performed on pure strains of Enterobacteriaceae isolated from different sampling points in order to determine their sensitivity to antibiotics. The method used was the disk diffusion technique (Kirby-Bauer), in agar medium with antibiotic-impregnated disks, following the recommendations of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (CA-SFM/EUCAST, 2024).

Selection of Antibiotics

The antibiotics tested on the Enterobacteriaceae were selected according to the CA-SFM/EUCAST (2024) guidelines. Table I shows the antibiotic families, the dosages of these antibiotics on the disks, and the interpretation categories based on the diameter of potential inhibition.

Table I: List of antibiotics used, along with their reference critical diameters for Enterobacteriaceae (CA-SFM/EUCAST, 2024).

Antibiotic family	Antibiotic	Codes	Dosage of antibiotic	Categories of interpretation and diameter of inhibition (in mm)		
				R	S	I
Bêtalactamines	Amoxicillin- cavulanic acid	AMC-30	30 µg	≤ 19	≥ 19	/
	Ceftazidime	CAZ-30	30 µg	≤19	≥22	19-21
	Cefotaxime	CTX-30	10 µg	≤20	≥20	/
	Cefepime	FEP-30	30µg	≤24	≥27	24-26

. R=resistance S=sensible I=intermediate

Preparation of the Medium and Bacterial Suspension

Medium Preparation

The medium used was Mueller-Hinton agar (Biorad), poured into Petri dishes. The agar thickness was approximately 4 mm. The surface of the agar was dried before use (CA-SFM/EUCAST, 2024).

Inoculum Preparation

Starting from an 18–24-hour culture on a non-selective agar medium (Plate Count Agar), a bacterial suspension in saline solution (0.9% NaCl) was prepared. Its turbidity matched the 0.5 standard on the McFarland scale, corresponding to a bacterial density of about 1×10^8 CFU/100 mL. This inoculum was then diluted 1:10 (1×10^7 CFU/100 mL) before seeding (CA-SFM/EUCAST, 2024).

Seeding, Disk Placement, and Incubation

The agar was seeded with the bacterial inoculum using the swabbing method. The entire agar surface was swabbed in three directions. Antibiotic disks were placed on the inoculated and dried agar surface, spaced 3 cm apart to avoid overlapping inhibition zones. Petri dishes were incubated within 15 minutes of disk placement at 37°C under aerobic conditions for 24 hours (CA-SFM/EUCAST, 2024).

Measurement of Inhibition Zones and Clinical Categorization

The diameters of circular inhibition zones were measured to the nearest millimeter using a caliper. Only diameters within quality control limits were considered. These diameters were interpreted based on critical thresholds provided by CA-SFM (2024) (Table VIII). Strains were then categorized clinically as resistant (R), sensitive (S), or intermediate (I). Percentage proportions of each clinical category were calculated relative to the total number of strains tested, using the following formulas:

% Resistance = (xr / t) × 100; **% Intermediate Sensitivity** = (xi / t) × 100, **% Sensitivity** = (xs / t) × 100.

Where: ***xr*** = number of resistant strains; ***xi*** = number of strains with intermediate sensitivity, ***xs*** = number of sensitive strains, ***t*** = total number of strains tested with the antibiotic (CA-SFM/EUCAST, 2024).

RESULTS AND DISCUSSION

RESULTS

Cultural characteristics of the colonies observed

The examination of bacterial colonies revealed various types. On standard agar medium, the colonies exhibited diversity in size, color, and morphological characteristics, mostly corresponding to Mesophilic Aerobic Heterotrophic Bacteria (MAHB) (Figure 3-A). On MacConkey agar medium, red colonies with diameters ranging from 1 to 2 mm presumptive of species from the genus *Serratia* and of medium size were observed (Figure 3-B1). Additionally, white colonies measuring between 1 and 2.5 mm presumptive of *Citrobacter* species were also identified (Figure 3-B2). On Salmonella-Shigella agar medium, colorless

S. marcescens (B1)

colonies with a black center presumptive of *Salmonella* species were likewise observed (Figure 3-C).

Figure 3: Photographs of observed bacterial colonies, A: MAHB (Mesophilic Aerobic Heterotrophic Bacteria); B1: various colonies of the genus *Serratia*; B2: colonies of the genus *Citrobacter*; C: colonies of the genus *Salmonella*. (Photo by Noah Ewoti, March 2025).

Identification of germs

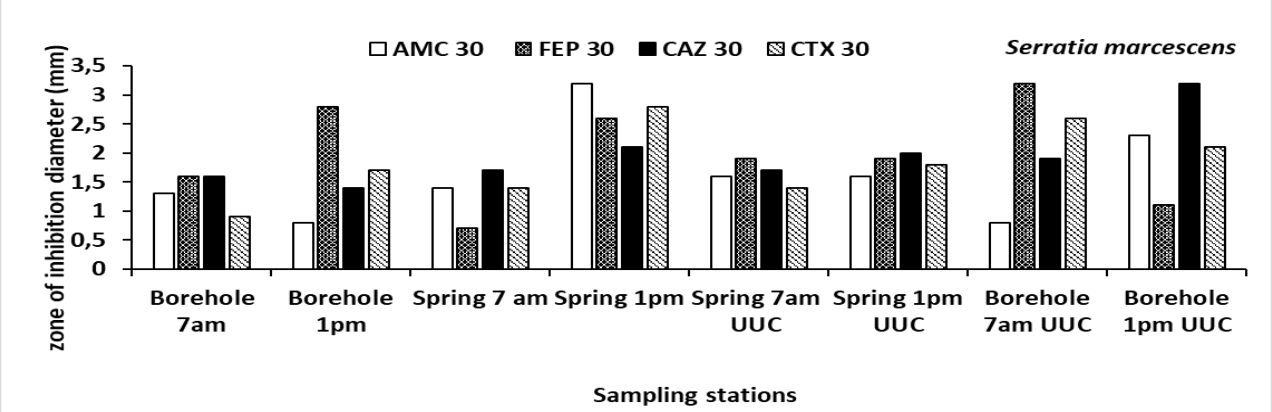
Cells from each colony isolated on MacConkey and Salmonella-Shigella (SS) agar were re-cultured in pure form on slanted agar in test tubes. From the suspensions prepared in 5 mL of sterile physiological water, various identification tests were performed. The results are presented in Table II. On MacConkey agar, medium-sized, spherical red colonies were identified as Gram-negative, oxidase-negative bacteria capable of fermenting glucose (Table II); these were determined to be *Serratia marcescens*. White colonies with slightly pink centers, of medium size and with similar biochemical characteristics to *S. marcescens*, were identified as *Citrobacter freundii* (Table I). On SS agar, small, colorless colonies with black centers, unable to ferment lactose and negative in the Simmons Citrate test, were identified as *Salmonella paratyphi*.

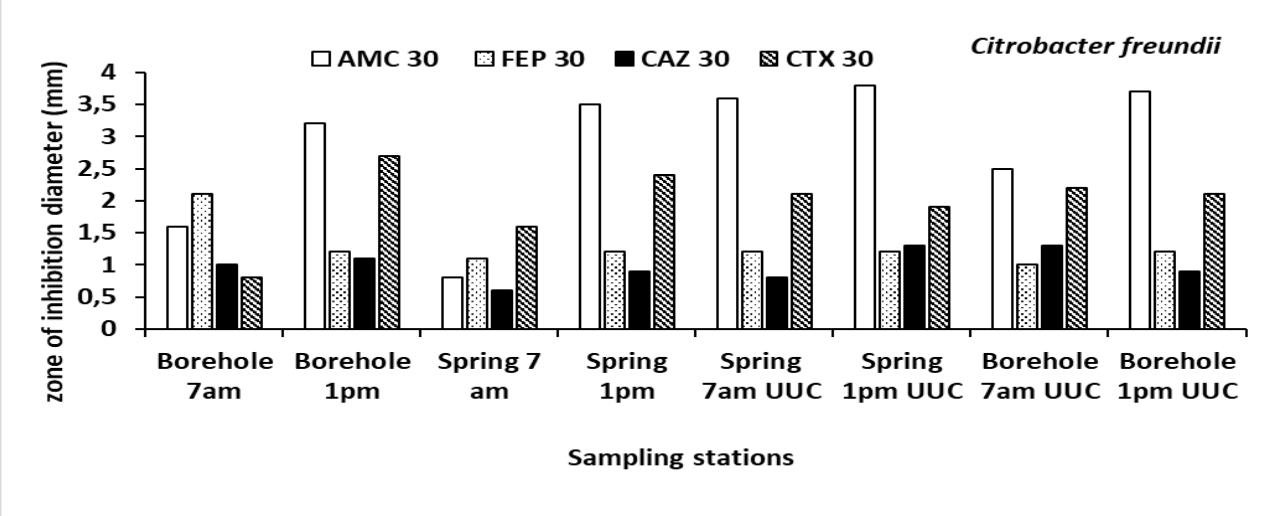
Table I: Identification Tests Conducted on the Various Isolated Genera

Identification Tests Performed	Bacterial Strains		
	Medium-sized red colony (B1)	Medium-sized white colony (B2)	Small colorless colony with a black center (C)
Gram's coloration	-	-	-
Glucose	+	+	+
Mobility	+	+	+
Fermentation of Mannitol	+	+	+
Lactose	-	+	-
H_2S	-	+	+/-
Gas production	+/-	+	+
Urea	+/-	-	-
Indole	-	-	-
Simmons citrate	+	+	-
Oxydase	-	-	-
Species	***S. marcescens***	***C. freundii***	***S. paratyphi***

Antibiotic susceptibility of Enterobacteriaceae

The susceptibility of Enterobacteriaceae to antibiotics in the β-lactam family varied depending on the sampling location where the species were isolated. For *Serratia marcescens*, the inhibition zones ranged from 32 mm to 7 mm. The largest zone (32 mm) was observed with cefepime (a β-lactam antibiotic) in the stored water sample collected at 7 am from the borehole. The smallest zone (7 mm) was also with cefepime, but in water sampled at 7 am from the spring. Concerning the *Citrobacter freundii,* the inhibition zone ranged from 38 mm to 6 mm. the largest zone of 38 mm was observed with amoxicillin clavulanic-acid in the stored spring water sample collected at 1pm while the smallest zone of 6 mm was observed with ceftazidime in the spring water collected at 7 am. The *Salmonella paratyphi* inhibition zone equally ranged from 38 mm to 6 mm, the 38 mm was observed with amoxicillin clavulanic-acid in two sampling station, the stored water collected from the borehole at 7 am and 1 pm. The 6 mm was equally observed with the amoxicillin clavulanic-acid but from water collected from the borehole at 1 pm. The variation in the different inhibition diameters for each species over time, indicating antibiotic sensitivity, is presented in Figure 4.





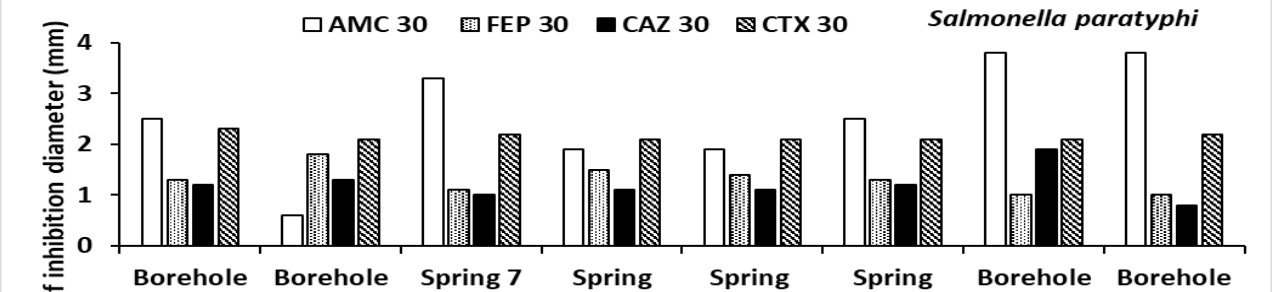


Figure 4: Diamètre d'inhibition montrant la Susceptibility of (A) *Serratia marcescens*, (B) *Citrobacter freundi*, (C) *Salmonella paratyphi* to antibiotics.

DISCUSSION

Presence of Pathogenic Bacteria in Collected Water Samples

The study reveals that both groundwater sampled directly at the source and groundwater collected from households under storage conditions contain pathogenic bacteria such as *Serratia marcescens*, *Citrobacter freundii*, and *Salmonella paratyphi*. These findings were consistent regardless of the time of sampling during the day. Similar observations regarding the presence of pathogenic bacteria in surface and groundwater in Yaoundé have previously been reported by Nola et al. (2000) and Moungang et al. (2013). The presence of these pathogens in water may be attributed to intense human activity, uncontrolled and chaotic urbanization in developing countries, characterized by the construction of pit latrines and the discharge of untreated wastewater into the environment due to malfunctioning or insufficient wastewater treatment facilities (Njiné et al., 2000; Noah Ewoti et al., 2025). Such practices can contaminate runoff and surface water, and through oblique infiltration or percolation, also affect groundwater (Nola et al., 2010). More recently, Baleng (2025) demonstrated the presence of pathogenic bacteria from the *Vibrio* and *Salmonella* genera in surface and groundwater in two localities of the Mbam region, near Yaoundé (Central Region, Cameroon). Despite this contamination, these water sources are still used for human consumption, as no formal water distribution company operates in these areas.

Sensitivity/Resistance of Isolated Bacteria from Collected Water

Furthermore, the present study shows that bacteria isolated from household-stored water samples, when compared to the critical diameters for Enterobacteriaceae (CA-SFM/EUCAST, 2024), exhibit resistance to beta-lactam antibiotics including cefepime, ceftazidime, the clavulanic acid–amoxicillin combination, and cefotaxime. These antibiotics function by inhibiting the synthesis of peptidoglycan, an essential component of the bacterial cell wall, leading to cell death. Additionally, they exert bactericidal effects by inhibiting penicillin-binding proteins (PBPs) (Ntsama et al., 2017).

Although these findings differ from those reported by Eheth et al. (2016) and Manouore Njoya et al. (2021; 2022), they align with results obtained by Baleng (2025), who observed that various *Salmonella* and *Vibrio* species isolated from water in the Mbam region were resistant to beta-lactams, quinolones, aminoglycosides, and sulfonamides. Some species exhibited multidrug resistance, which the author attributes to bacterial exposure to pesticide residues and heavy metals from agricultural inputs. Groundwater sources in developing countries are often heavily exploited by local populations for market gardening.

CONCLUSION

The present work aims to evaluate antibiotic sensitivity of bacteria from underground water points in source and storage water in household in Yaounde (Centre Cameroon). in addition to the MAHB, the

waters sampled in the households and in the water supply sources harbor pathogenic bacteria such as *Serratia marcescens*, *Citrobacter freundii*, and *Salmonella paratyphi*. The microbial group under investigation exhibited resistance to amoxicillin-clavulanic acid, ceftazidime, cefotaxime, and cefepime. This result suggests that the contamination may be due to storage conditions. According to WHO standards, the waters of certain points are not recommended for consumption human without any treatment prior

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

Author's contributions

Olive Vivien Noah Ewoti, conceptualized, analyzed the data and prepared the manuscript. Nene Zakiyatou, Lucie Leme Banock, Ladibé Pélagie, Bolivar Ndourwé and Moise Nola in collecting data, in analyzing and interpreting. The was supervised by Olive Vivien Noah Ewoti. All authors have read, agreed and approved the final manuscript.

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