

Profile and Antibiotic Resistance of Some Pathogenic Enterobacteria Isolated from Effluents from Social Housing in the City of Yaoundé-Cameroon

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Abstract

The increase in bacteria resistant to chemical agents, particularly with regard to antibiotics, is one of the world's greatest health scourges. Among these bacteria are enterobacteria, which are frequently found in hospital and agricultural effluents, but also in domestic effluents. The aim of this study was to assess the abundance and resistance profile of some pathogenic enterobacteria isolated in effluents from social housing in the city of Yaoundé. Wastewater samples were collected at the discharge points of effluents in SIC camps in certain neighbourhoods of Yaoundé. The germs were isolated on *Salmonella-Shigella* medium by the surface spreading technique and then identified using standard biochemical techniques. Antibiotic susceptibility testing of enterobacteria isolated was done using the antibiotic disc diffusion method on Müller-Hinton agar. Physicochemical parameters were analyzed using appropriate techniques. Regarding bacterial abundance, a total of 371.45×10^5 CFU/mL belonging to the different genera of isolated enterobacteria was obtained, which enabled the identification of three species, namely *Shigella dysenteriae*, which was the most prevalent with a percentage of 55%, followed by *Salmonella typhi* (30%), and finally *Proteus vulgaris* (15%). The prevalence of antibiotic resistance varied from spe-

cies to species in the different sampling points according to the season. Antibiotic susceptibility tests showed that in the dry season, the highest resistance rate was that of *Proteus vulgaris* (75%), while in the rainy season, the highest resistance rate was that of *Shigella dysenteria* (71%). Overall, the enterobacteria strains were resistant to several antibiotics used, such as amoxicillin, ampicillin, cefotaxime, penicillin, ciproflaxacin, and azithromycin, thus demonstrating the multi-resistant nature of these strains, particularly *Salmonella typhi* and *Proteus vulgaris*, for which the prevalence rate was 100 %. Physicochemical analyses showed that these effluents were alkaline ($\text{pH} > 7.7$), highly mineralized (electrical conductivity $1190.6\mu\text{S}/\text{cm} \pm 644.04 \mu\text{S}/\text{cm}$), and had a low temperature variation ($25.75^\circ\text{C} \pm 1.65^\circ\text{C}$). Domestic effluents could be hotspots for the dissemination of resistant pathogenic enterobacteria, requiring optimisation of purification processes and increased monitoring to limit environmental contamination.

Keywords

Domestic Effluents, Pathogenic Enterobacteria, Abiotic Factors, Antibiotic, Resistance Profile

1. Introduction

Wastewater, also known as liquid effluent, is water that has undergone physical, chemical, and biological changes after human use. The impact of wastewater is significant. On the one hand, it poses a health risk and, on the other, it threatens the environment due to the presence of pathogens such as bacteria, viruses, and parasites. This polluted water is discharged into the environment after use due to poorly developed or even non-existent wastewater collection and treatment systems. Effluent discharges often lead to the contamination of natural aquatic environments and, as a result, to an increase in the incidence of waterborne diseases (Garcia-Armisen et al., 2011).

Wastewater is a reservoir for pathogenic microorganisms such as parasites, bacteria, and even viruses; through certain mechanisms, many infectious germs are transmitted and cause high human mortality (Tamsa Arfao et al., 2021). These microorganisms cause diseases such as cholera, gastroenteritis, and typhoid fever, among others. The presence of these potential pathogens can therefore pose a serious threat to public health, highlighting the need for resource management (Pulchérie et al., 2012). Among the most dangerous agents are Enterobacteria, which are Gram-negative, facultative aerobic-anaerobic bacteria found everywhere in soil, water, and especially in the intestines of humans and animals (Manouore Njoya, 2023). A significant proportion of the bacteria isolated in fresh water, in most effluents from municipalities, industries, veterinary activities, hospitals, and secondary treatment sources belong to the Enterobacteriaceae family (Harris et al., 2014). These bacteria are most commonly involved in human infectious diseases, the treat-

ment of which requires the use of antibiotics (Savadago & Boubkeri, 2016).

However, the resistance of Enterobacteriaceae to antibiotics is undergoing a worrying global evolution, with an increasing impact of extended-spectrum beta-lactamases (ESBLs), which are spreading particularly in community settings (Belmonte et al., 2010). Enterobacteria develop several forms of antibiotic resistance, the main one being the secretion of inhibitory enzymes (Kulkarni et al., 2015). Previously, resistant germs were found in hospitals, but today they are increasingly detected in the environment, with no particular link to healthcare facilities (Kumar & Schwezer, 2005). Their presence in the environment is due more to the increase in the frequency of human carriage of antibiotic-resistant bacteria, which promotes the exchange and spread of resistance genes via wastewater that can contaminate surface water and groundwater (Machado et al., 2013). Resistance genes can also originate from antibiotic-producing microbes or those that coexist with them in the environment (D'Costa et al., 2011).

Previous studies have shown that municipal and hospital effluents, secondary treatment sources, surface water, and groundwater harbour Enterobacteriaceae (Harris et al., 2014). Numerous studies on the impact of abiotic factors on antibiotic resistance have shown that certain parameters, such as temperature and pH, may influence the antibiotic resistance of germs in aquatic environments (Manouore Njoya, 2023). In addition, studies conducted in hospital wastewater in Cameroon have shown that many bacteria, particularly Enterobacteriaceae and *Staphylococci*, exhibit resistance profiles to several families of antibiotics, such as β -lactams and quinolones (Ebongue et al., 2018).

Despite this research, little information is available on Enterobacteriaceae isolated from effluents from social housing in the city of Yaoundé. In addition, very little data is available on the assessment of antibiotic resistance in bacterial species isolated from wastewater from these dwellings. The present study, therefore, aims to evaluate the antibiotic resistance profile of some pathogenic enterobacteria isolated from effluents from social housing in the city of Yaoundé.

2. Materials and Methods

2.1. Description of the Study Area and Sampling Locations

The study was carried out in the city of Yaoundé, capital of the Centre Region of Cameroon, located at 300 Km from the Atlantic coast between latitude 3° 5' North and Longitude 11° 31' East. After the prospection, seven social housing sites were selected based on specific criteria, including ease of access, population density, type of sanitation system, configuration of latrine pits, mode of effluent discharge into the environment, and spatial distribution. These criteria were applied to ensure the representativeness of various wastewater management practices and discharge conditions observed throughout the city's social housing network. Wastewater samples were collected from seven designated points: MDG, RPE, BIY, Cverte, MSI, MSII, and MFD. Those geographic coordinates are given in **Table 1**.

Table 1. Geographic coordinates of the sampling stations, sampling point descriptions, and observations.

Sampling Point Codes	SICS Camp Names	Geographic Coordinates			Sampling Point Description and Observation
		Latitude (N)	Longitude (E)	Altitude (m)	
MDG	Mendong	03°83'60.48"	011°46'92.93"	782	Located in Mendong at building L of the SIC estate, this point is a medium flow pit. It is more or less equipped.
RPE	Rond Point Express	03°83'67.89"	011°48'43.80"	715	Located in Rondpoint Express, 100m away from the SIC Medong. The wastewater overflows the pit and moves through the environment at a medium speed.
BIY	Biyem-Assi	03°83'87.50"	011°48'60.85"	697	It is located on the site of the Biyem-Assi wastewater treatment plant.
Cverte	Cite-Verte	03°87'62.19"	011°48'94.59"	764	Located at Cite-Verte, opposite the hospital, wastewater is fed into a tank. It flows at a medium speed.
MSI	Messa I	03°87'17.10"	011°50'71.91"	733	Located at Messa, not far from the Church. Sewage is discharged into an overflow tank and, due to the high flow rate, the effluent is discharged directly into the environment.
MSII	Messa II	03°87'12.26"	011°50'68.95"	733	Wastewater is discharged into a pit; the average wastewater flow is.
MFD	Mfandena	03°88'57.00"	011°54'92.50"	756	Located at Mfandena, just behind the Mfandena SIC camp. Wastewater from this camp flows into a gully with an average flow rate of.

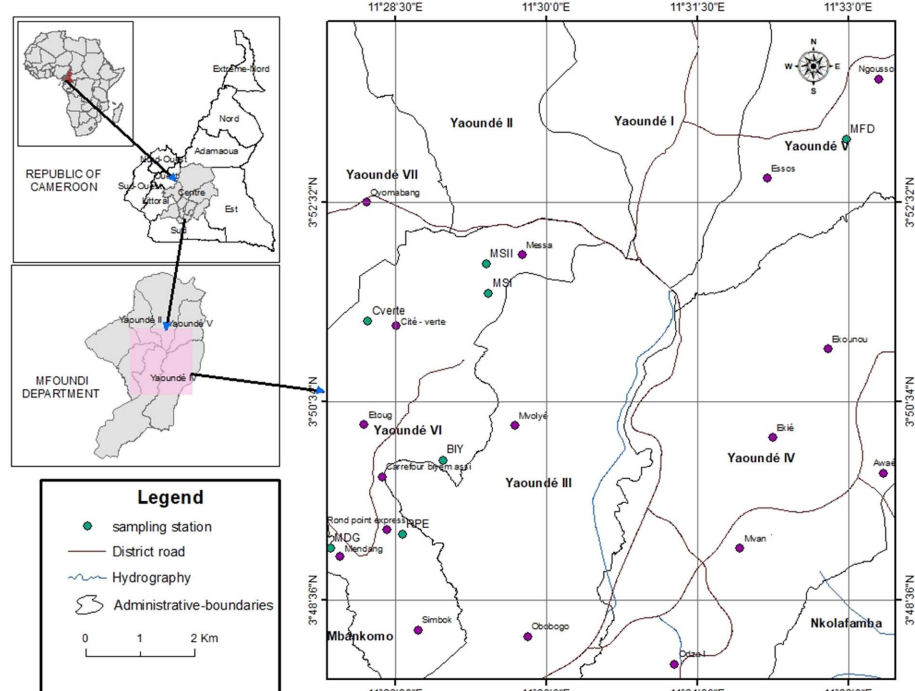
**Figure 1.** Map of the study area showing sampling stations.

Figure 1 shows the geographical location of the study area and the sampling

stations.

2.2. Environmental Sampling Approaches and Parameter Assessment

The samples for the bacteriological analysis were collected in sterile glass bottles of 500 mL. Samples for physicochemical analyses were collected bubble-free by a diver in 250 mL double-capped polyethylene bottles following the techniques recommended by some authors (Rodier et al., 2009). At the level of each sampling station, the physico-chemical analysis focused on 12 variables. Physico-chemical parameters (water Temperature ($^{\circ}\text{C}$), pH, electrical conductivity of the water ($\mu\text{S}/\text{cm}$), salinity (ppm), suspended solids (mg/L), water colour (Pt. Co), turbidity (FAU), nitrates (mg/L), nitrites (mg/L), orthophosphates (mg/L PO_4^{3-}) and ammonium ions (mg/L NH_4^+) were measured by colorimetry and/or spectrophotometry according to the techniques described by some authors (Rodier et al., 2009; Tamsa Arfao et al., 2021). The various samples were then transported to the laboratory in a refrigerated chamber to undergo the various analyses and tests required. Bacteriological analysis begins within 4 hours of sampling.

2.3. Bacterial Isolation and Identification Procedures

The isolation of the target genera was carried out using the surface spread method, which consists of first taking 100 μL using a sterile micropipette, then depositing the volume of water taken on the surface of the *Salmonella-Shigella* (SS) agar previously poured into sterile 90 mm diameter Petri dishes. The inoculum was distributed uniformly across the surface using a sterile rake or spreader until complete absorption was achieved. This exercise was carried out under sterile conditions ensured by the Bunsen burner flame (Benzerfa et al., 2021). Subsequently, the plates were subjected to an incubation process at a temperature of 37°C for a duration of 24 hours. Bacterial strains with satisfactory characteristics were counted using the direct counting method (Holt et al., 2000) and the results were expressed in decimal logarithmic units (Log_{10} (CFU/mL)) of water analysed to avoid large variations during the sampling period (Marchal et al., 1991) and also to better represent the variation and limit of the significant differences between the densities of the bacteria sought.

The identification involved first conducting a macroscopic examination, followed by a microscopic examination, and finally biochemical tests. Macroscopic observation consisted of examining the cultural characteristics of bacterial colonies on the surface of different culture media. The cultural characteristics considered were colour, size, contours, colony surface configuration, and appearance (Denis et al., 2011). Microscopic observation of fresh presumptive colonies allowed their mobility or immobility to be observed. Microscopic observation after Gram staining revealed the nature of the bacterial cell wall, their shapes, and their modes of grouping (Denis et al., 2011). Bacterial species were identified using standard tests. These tests included testing for oxidase, catalase, glucose fermentation, gas production, oxygen affinity (facultative aerobic/anaerobic), motility,

mannitol, lactose fermentation, H₂S production, urease, citrate, among others (Holt et al., 2000).

2.4. Antimicrobial Susceptibility Tests

After the biochemical identification tests, the colonies were transferred to slanted PCA medium. After 24 hours of incubation, pure bacterial cells were harvested using a sterile platinum loop and placed in a test tube containing 10 mL of sterile physiological water. The homogenised bacterial suspension was vortexed and adjusted to a density of 0.5 MacFarland (BaCl₂ and H₂SO₄ 1%), corresponding to a bacterial concentration of approximately 1.5×10^8 CFU/mL. This concentration was determined by reading the optical density (OD) on a DR/2800 spectrophotometer at 625 nm (CA-SFM, 2023). Antimicrobial susceptibility testing was performed on pure strains in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2023). Bacterial suspensions were uniformly inoculated onto the entire surface of Mueller-Hinton agar plates (4 mm thickness) using sterile cotton swabs. The inoculated dishes were dried for 15 minutes at room temperature under aseptic conditions. Subsequently, four antibiotic discs were aseptically placed and gently pressed onto the surface of the agar to ensure complete contact. The plates were then incubated in an aerobic atmosphere at 37°C for 18 - 24 h, after which inhibition zone diameters were measured and interpreted. The antibiotics used belonged to several families. They were in the form of a disc of blotting paper, 6 mm in diameter, impregnated with well-determined quantities of active substances and rigorously controlled for the diffusion method. A total of eight antibiotics representative of major antimicrobial classes routinely employed in the treatment of infections caused by pathogenic Enterobacteriaceae were selected. The selection was based on their widespread clinical use in hospital settings. These included Beta-lactamin, macrolides, quinolones, and aminoglycoside antibiotics. Table 2 lists the antibiotics used, their groups, and their characteristics.

Table 2. Antibiotics, disc loads, and critical reference diameters for Enterobacteriaceae (CA-SFM, 2018; 2023).

Antibiotic Families	Standards	Antibiotics Tested	Antibiotic Concentration (µg)	Critical Reference Diameter (mm)		
				Sensitive	Intermediate	Resistant
Beta-lactamin	CA-SFM (2018)	Penicillin G (P10)	10	≥29	-	<29
Beta-lactamin		Cefotaxim (CTX30)	30	≥26	23 - 25	≤22
Cyclines		Tetracycline (TE30)	30	≥19	-	<17
Beta-lactamin	CA-SFM (2023)	Amoxicillin (AX20)	20	≥19	-	<19
Beta-lactamin		Ampicillin (AM10)	10	≥14	-	<14
Aminosides		Gentamicin (GEN10)	10	≥17	-	<17
Macrolides		Azithromycin (AZM15)	15	≥12	-	≤12
Quinolones		Ciprofloxacin (CIP5)	5	≥25	22 - 24	<22

After incubation, the reading was taken by measuring the diameters of the inhibition zones using a caliper in accordance with CA-SFM recommendations (CA-

SFM, 2018; 2023), to declare the germ sensitive, intermediate, or resistant in relation to the critical diameters of the antibiotic tested. The results yielded three clinical categories: sensitive (S), resistant (R), and intermediate (I). The sensitive (S) category means that the strain is susceptible to the antibiotic treatment given at the normal dose. In the intermediate category (I), the success of the therapy is not predictable. If the antibiotic is used at a higher dose than usual, the treatment will be effective. Intermediate means moderately susceptible or moderately resistant. The resistant (R) category means that there is a high probability of treatment failure regardless of the type of antibiotic treatment, or that the strain will not be affected regardless of the type of treatment.

2.5. Data Analysis

Histograms plotted using Microsoft Excel 2016 were used to illustrate the temporal evolution of the density of the different bacterial species studied, as well as the percentages of antibiotic sensitivity. A double-axis histogram (or dual-axis chart) was used to represent two different physicochemical variables on the same graph. SPSS 25.0 software was used to calculate Spearman's rank correlation coefficient "r". Correlations between biological and abiotic variables were established using this coefficient. To characterize the sampling stations based on all the physicochemical parameters measured and the biological affinities at different stations throughout the study, principal component analysis (PCA) was performed using XL STAT 2016 software. The purpose of this descriptive factorial statistical method is to represent as much information as possible in a large data table in graphical form (Tamsa Arfao et al., 2021).

3. Results and Discussion

3.1. Biochemical Characterization of Isolates

Table 3. Identification tests were carried out, and different species were isolated.

Biochemical Test	Colony Color		
	Pink Colonies	Colourless Colonies	Opaque Colonies with Black Center
Catalase	±	+	+
Glucose	+	+	+
Lactose	-	-	-
H ₂ S	-	+	+
Gas	-	+	-
Mannitol	+	-	+
Mobility	-	+	+
Citrate	+	+	+
Urease	-	+	-
Indole	±	±	-
Bacteria Species	<i>Shigella dysenteria</i>	<i>Proteus vulgaris</i>	<i>Salmonella typhi</i>

+: Positive for 90 to 100% of strains; -: Negative for 90 to 100% of strains; ±: variable.

The pathogenic enterobacterial germs sought were isolated in effluents from a few social housing units in the city of Yaoundé. Their characteristics on *Salmonella-Shigella* medium were as follows: opaque colonies with black centres, varying in size between 2 and 3 mm, presumed to be *Salmonella* sp., small pink colonies, presumed to be *Shigella* sp., and colourless and transparent colonies between 2 and 3 mm, presumed to be *Proteus* sp. The biochemical tests carried out using the API 20E system from the colonies isolated made it possible to have the biochemical profile of the strains studied, as presented in **Table 3**.

3.2. Spatio-Temporal Variations of the Bacteria

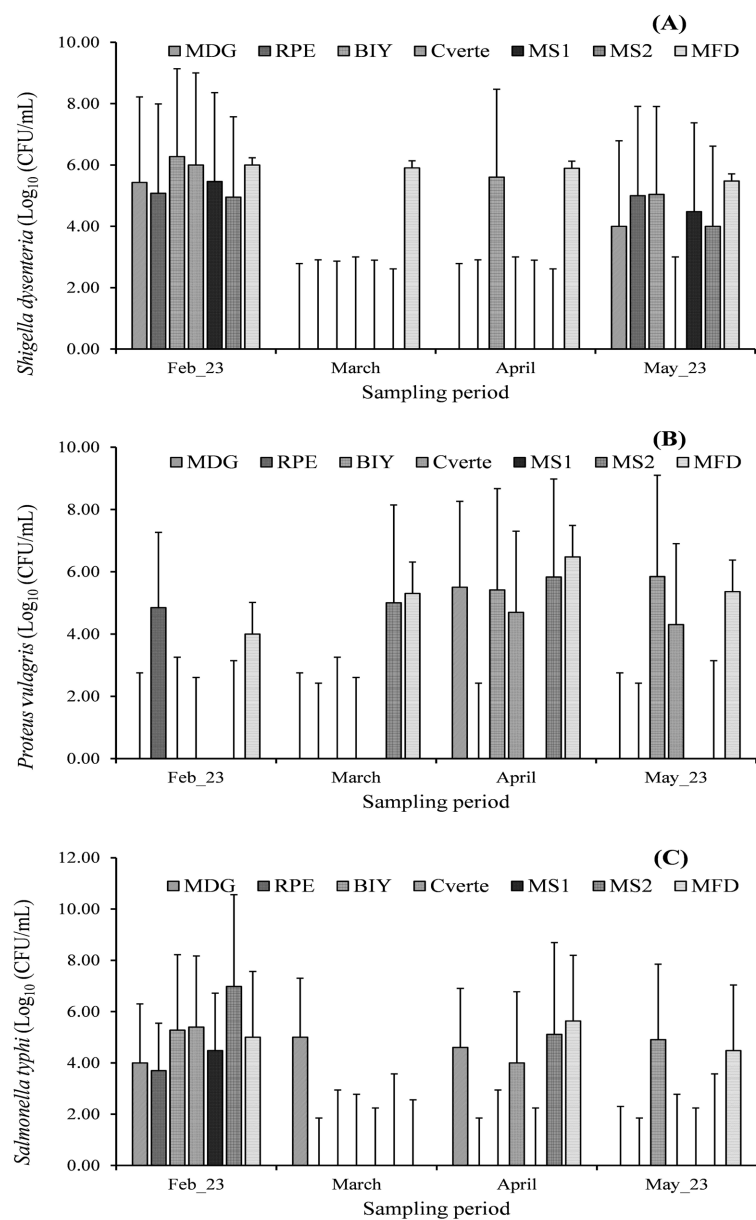


Figure 2. Spatio-temporal variations in cell abundance of (A) *Shigella dysenteriae*, (B) *Proteus vulgaris*, (C) *Salmonella typhi*.

The distribution of the abundances of the isolated bacterial species varied from one sampling point to another and from one month to another. During the study period, the highest density of *Proteus vulgaris* was recorded at the MFD point in April (6.48 Log₁₀ CFU/mL) and reached a value of zero during all sampling months at the MS1 point. The lowest value (0 Log₁₀ CFU/mL) of *Shigella dysenteriae* was recorded at the RPE, Cverte, MS1, and MS2 points in April, May, and March, respectively. The highest value (6.59 Log₁₀ CFU/mL) was recorded at the Cverte point in March. The densities of *Salmonella typhi* varied between 0 Log₁₀ CFU/mL (at all sampling points) and 6.98 Log₁₀ CFU/mL (at point MSII in February) (Figure 2).

3.3. Antimicrobial Susceptibility Patterns among Bacterial Strains Isolated

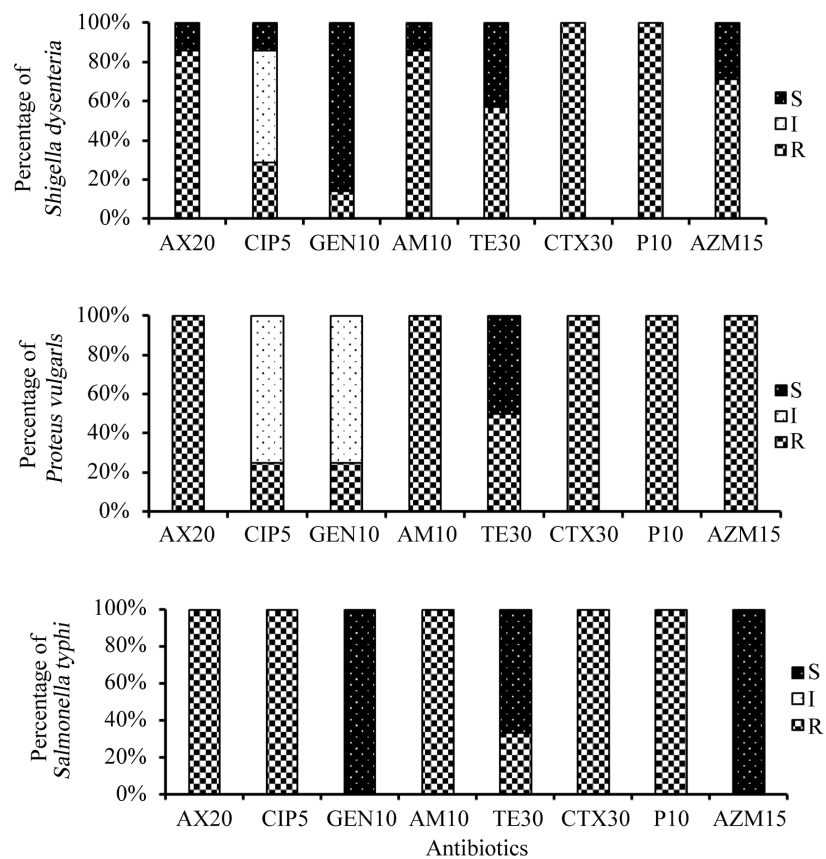


Figure 3. Distribution of the percentages of sensitivity/resistance of bacteria to antibiotics during the dry season (AX20 = Amoxicillin20, CIP5 = Ciprofloxacin, GEN10 = Gentamicin10, AM10 = Ampicillin10, TE30 = Tetracycline30, CTX30 = Cefotaxime30, P10 = Penicillin10, AZM15 = Azithromycin15).

The antibiotic sensitivity/resistance of the three species from the sampling points was assessed. During the dry season, *Proteus vulgaris* and *Salmonella typhi* species showed 100% resistance to 5 of the 8 antibiotics tested (amoxicillin, ampicillin, cefotaxime, penicillin, and azithromycin for *Proteus vulgaris* and amoxicillin, am-

picillin, ciprofloxacin, penicillin, cefotaxime for *Salmonella typhi*). Meanwhile, *Shigella dysenteriae* species showed 100% resistance to 2 antibiotics (cefotaxime and penicillin). **Figure 3** shows the sensitivity/resistance distributions of each bacterial species to each antibiotic during the dry season. During the rainy season, *Proteus vulgaris* showed 100% resistance to three antibiotics (ampicillin, tetracycline, and penicillin), while *Shigella dysenteriae* showed 100% resistance to four antibiotics (amoxicillin, tetracycline, penicillin, and ampicillin) and *Salmonella typhi* to five antibiotics (amoxicillin, tetracycline, penicillin, ampicillin, and ciprofloxacin). In general, the isolated bacterial species were more resistant in the dry season than in the rainy season. **Figure 4** shows the sensitivity/resistance distributions of each bacterial species to each antibiotic during the rainy season.

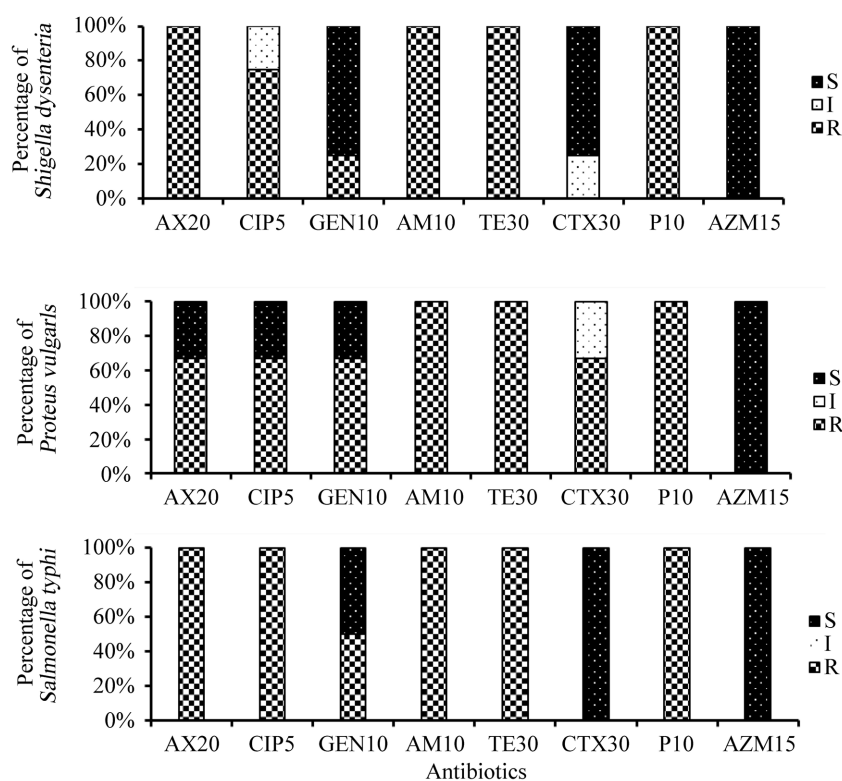


Figure 4. Distribution of percentages of bacterial sensitivity/resistance to antibiotics during the rainy season (AX20 = Amoxicillin20, CIP5 = Ciprofloxacin, GEN10 = Gentamicin10, AM10 = Ampicillin10, TE30 = Tetracycline30, CTX30 = Cefotaxime30, P10 = Penicillin10, AZM15 = Azithromycin15).

3.4. Physico-Chemical Variables

Temperature values fluctuated between 20.8°C and 27.7°C with an average value of 25.75°C ± 1.65°C. The highest value was recorded at point MS1 in April, and the lowest value at point RPE in February (**Figure 5(A)**). With regard to pH (basic), values fluctuated between 6.65 and 8.63 UC, with an average value of 7.74 UC ± 0.51 UC. The highest value was recorded at the MDG point in March, and the lowest value was obtained at the MFD point in April (**Figure 5(A)**). Turbidity values fluctuated

tuated between 2 and 9600 FAU, with an average value of $1018.25 \text{ FAU} \pm 1882.16 \text{ FAU}$. The highest value was recorded at point MFD in February, and the lowest value at point MDG in May. In addition, water color values ranged from 9 to 18,688 pt.co with an average value of $3100.2 \text{ pt.co} \pm 4138.7 \text{ pt.co}$. The highest value was recorded at the MFD point in February, and the lowest value at the MDG point in May (**Figure 5(B)**). The MES values fluctuated between 0 and 5928 mg/L with an average value of $579 \text{ mg/L} \pm 1139.6 \text{ mg/L}$. The highest value was recorded at the MFD point in February, and the lowest value at the MDG point in May. In addition, TDS values ranged from 129 to 1550 ppm with an average value of $594.1 \text{ ppm} \pm 321.6 \text{ ppm}$. The highest value was recorded at the BIY point in February, and the lowest value at the MDG point in May (**Figure 5(C)**).

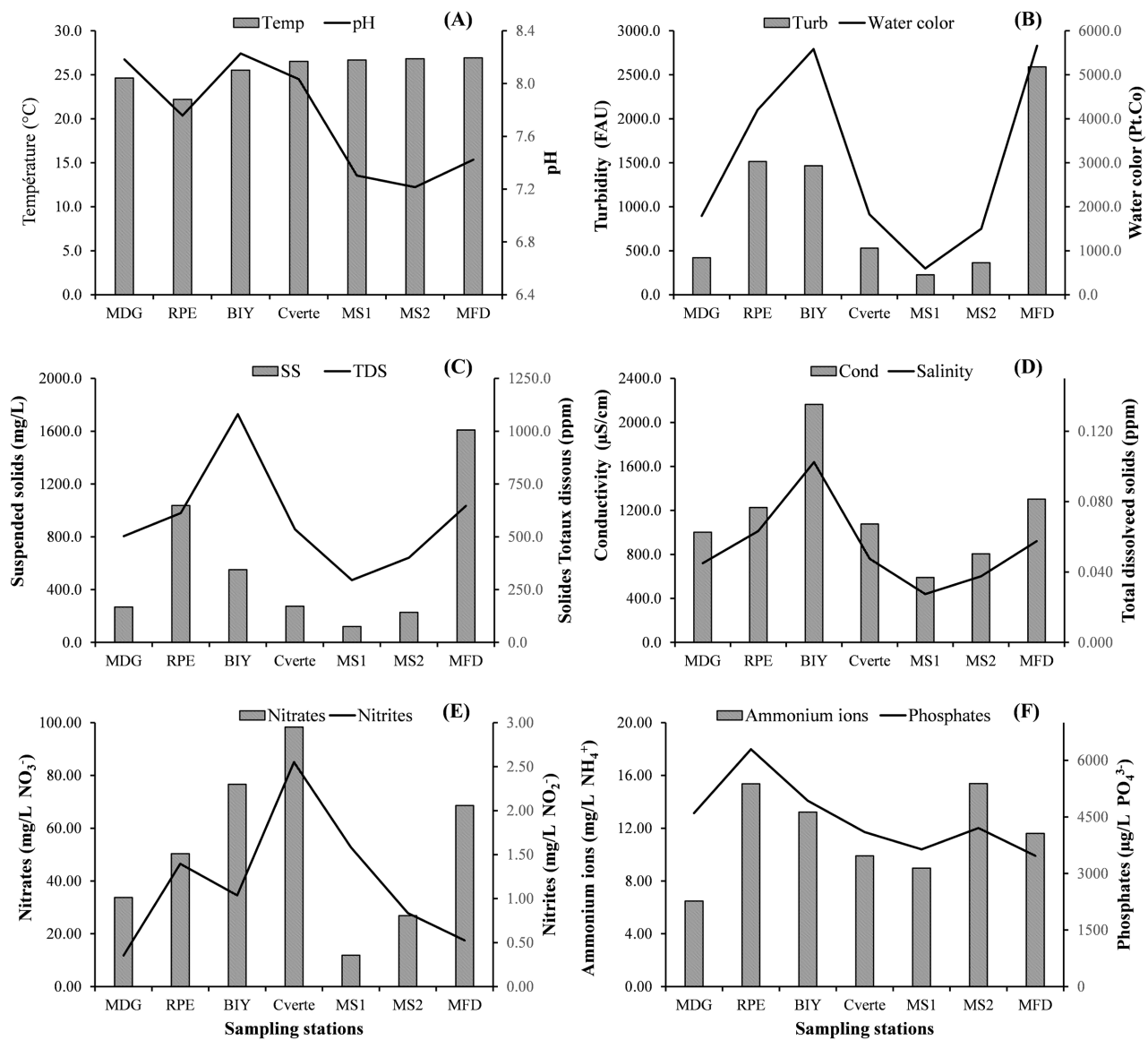


Figure 5. Spatial variation of the mean values of (A) temperature & pH, (B) turbidity & water color, (C) suspended solids & total dissolved solids, (D) electrical conductivity & salinity, (E) nitrates & nitrites, and (F) ammonium ions & phosphates.

Electrical conductivity values ranged from 253 to 3100 $\mu\text{S}/\text{cm}$, with an average value of $1190.6 \mu\text{S}/\text{cm} \pm 644.04 \mu\text{S}/\text{cm}$. The highest value was recorded at the MDG point in May, and the lowest value was obtained at the BIY point in May. In addition, salinity values fluctuated between 0.01 and 0.15 ppm, with an average value of $0.05 \text{ ppm} \pm 0.03 \text{ ppm}$. The highest value was recorded at point BIY in February, and the lowest value was obtained at point MDG in May (**Figure 5(D)**). Nitrates ion values fluctuated between 0.6 and 216 mg/L, with an average value of $45.05 \text{ mg/L} \pm 53.2 \text{ mg/L}$. The highest value was recorded at point MFD in February, and the lowest value was obtained at point MDG in May. In addition, nitrite ion values ranged from 0.003 to 348 mg/L, with an average value of $40.09 \text{ mg/L} \pm 89.77 \text{ mg/L}$. The highest value was recorded at the BIY point in February, and the lowest value was obtained at the MDG point in May (**Figure 5(E)**). Ammoniacal nitrogen values fluctuated between 1.62 and 1408 mg/L, with an average value of $60.95 \text{ mg/L} \pm 264.13 \text{ mg/L}$. The highest value was recorded at the RPE point in February, and the lowest value was obtained at the MDG point in April. In addition, phosphate ion values ranged from 146 to 9888 mg/L, with an average value of $4475.07 \text{ mg/L} \pm 3036.55 \text{ mg/L}$. The highest value was recorded at the BIY point in February, and the lowest value was obtained at the MS1 point in May (**Figure 5(F)**).

3.5. Influence of Abiotic Environmental Factors on Bacterial Abundance

Spearman's correlations between the physicochemical and bacteriological parameters and between the bacteriological parameters revealed several significant positive or negative relationships at the 1% and 5% thresholds. Correlations between abiotic parameters and the densities of isolated bacteria were performed using Spearman's "r" correlation test. This test revealed highly significant ($p < 0.01$) and positive correlations between electrical conductivity, TDS, water color, salinity and the species *Shigella dysenteria* ($r = 0.589$; 0.583 ; 0.499 and 0.545) on the one hand, and on the other hand, significant ($p < 0.05$) and positive correlations were recorded between pH, turbidity, suspended solids, nitrates, nitrites and *Shigella dysenteria* ($r = 0.403$; 0.419 ; 0.378 ; 0.457 and 0.382). In addition, an increase in nitrites leads to a significant increase ($p < 0.05$) in the cell densities of *Salmonella typhi* ($r = 0.404$). **Table 4** summarises the various correlations.

The comparison between the abiotic and microbiological variables during the study period was performed using the Kruskal-Wallis H test. This test shows, on the one hand, that spatially, all species are identical at all sampling points with a significance level of 0.05. On the other hand, in temporal terms, *Proteus vulgaris* and *Shigella dysenteria* remained identical throughout the sampling study period, while *Salmonella typhi* varied significantly during the sampling months at a significance level of 0.05. The Principal Component Analysis (PCA) applied to the various biological and physicochemical variables shows a grouping of parameters into two clusters (**Figure 6**). In this factorial design, cluster 1 (N1) includes the MFD point, in which *Salmonella typhi*, *Proteus vulgaris*, and *Shigella dysenteria* have strong affinities with ammoniacal nitrogen, turbidity, suspended solids, TDS,

water color, electrical conductivity, and nitrate ions. Cluster 2 (C2) contains the BIY and RPE points, where there are strong affinities between salinity, nitrite ions, pH, and phosphate ions. **Figure 6** illustrates the affinities between bacterial abundances and abiotic variables.

Table 4. Correlations between bacteriological and physico-chemical variables in wastewater from SIC camps in Yaoundé.

Physico-Chemical Variables	Bacteriological Variables		
	<i>Proteus vulgaris</i>	<i>Shigella dysenteria</i>	<i>Salmonella typhi</i>
Temperature	0.193	-0.038	0.162
pH	-0.031	0.403*	-0.076
Electrical Conductivity	0.171	0.589**	0.210
Turbidity	0.024	0.419*	0.141
TDS	0.167	0.583**	0.224
Suspended Solid	0.163	0.378*	0.120
Water Color	0.103	0.499**	0.126
Salinity	0.153	0.545**	0.125
Ammoniacal Nitrogen	-0.282	0.189	-0.035
Phosphates	-0.322	0.092	0.306
Nitrates	-0.047	0.457*	0.246
Nitrites	-0.012	0.382*	0.404*

*: Significant correlation $p < 0.05$; **: Very significant correlation $p < 0.01$.

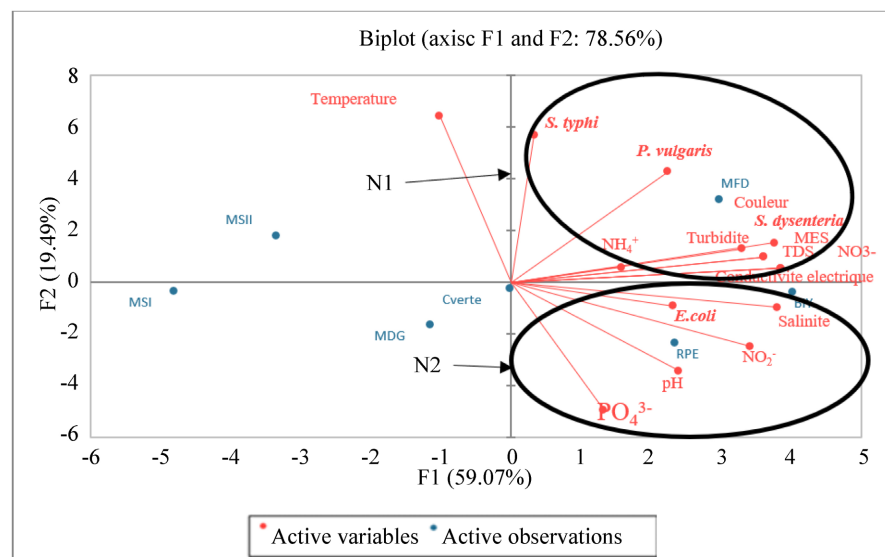


Figure 6. PCA grouping affinities between bacterial abundances and abiotic variables.

4. Discussion

Bacteriological analyses show that the sampled waters harbour bacterial commu-

nities classified as strict pathogens. Some Enterobacteriaceae, namely the genera *Salmonella*, *Shigella*, and *Proteus*, were found in abundance in almost all of the water sampling points. These results are similar to those of other researchers who demonstrated that these bacteria are very abundant in the aquatic environment (Mendaci & Mihoubi, 2015). Studies in the city of Yaoundé showed that wastewater, surface water, and groundwater harbour bacteria of the genera *Salmonella*, *Shigella*, and *Proteus*, among others (Manouore Njoya, 2023). These species are known as indicators of faecal contamination, so the high abundance of *Salmonella typhi*, *Shigella dysenteria* and *Proteus vulgaris* recorded at various wastewater points could be explained by the presence of faecal matter in the hospital and community waste they receive (Bouteleux, 2005). Hospital and community discharges are heavily laden with faecal matter, which causes the proliferation of Enterobacteriaceae (Ameziane & Benaabidate, 2014). Overall, the abundance of *Shigella dysenteria* was higher during February and March, corresponding to the dry season. Indeed, during the dry season, temperatures appear to be ideal for the growth of these bacteria. These results corroborate several previous studies showing high abundances of Enterobacteriaceae during the dry season and low abundances during the rainy season.

Antibiograms performed with isolates of *Salmonella typhi*, *Shigella dysenteria* and *Proteus vulgaris* show a multitude of multi-resistant strains depending on the season and the antibiotic present. A higher resistance rate was also observed in the dry season (75%) than in the rainy season (71%). Variable resistance rates over different months were also found by some authors, whose results revealed seasonal variation in antibiotic-resistant bacteria in river water in central India (Akiyama & Savin, 2010; Diwan et al., 2018). This can be explained by the fact that during this period, microorganisms are faced with harsh climatic conditions, forcing them to develop mechanisms that enable them to survive during this season. However, all isolates showed 100% resistance to ampicillin, tetracycline, and penicillin in the rainy season, to cefotaxime and penicillin in the dry season, and to penicillin in both seasons. These very high percentages among the species could be explained, on the one hand, by the fact that Enterobacteriaceae produce β -lactamase, which inhibits the action of antibiotics (Garneau-Tsodikova & Labby, 2016) and, on the other hand, by the fact that Enterobacteriaceae have a clear ability to acquire and exchange genes carrying resistance factors, and the intestinal flora provides an extraordinary opportunity for the circulation of genetic information between bacteria (Brahmia et al., 2013). In fact, enterobacteria are multi-resistant to beta-lactams, which are antibiotics commonly used in veterinary and bacterial medicine in humans, particularly ampicillin, which is also used in the treatment of various bacterial infections in humans. This multi-resistance can be explained by the presence in the environment of bacteria harbouring resistance genes derived from the misuse of these drugs in humans and animals. The observed 100% resistance to Penicillin among Enterobacteriaceae isolates is consistent with their well-documented intrinsic resistance to this antibiotic. Members

of the Enterobacteriaceae family possess an outer membrane with low permeability and frequently express chromosomally encoded β -lactamases, which confer natural resistance to narrow-spectrum penicillins such as Penicillin G (Livermore, 1995; Jacoby & Munoz-Price, 2005). Therefore, this result reflects an expected intrinsic resistance phenotype rather than acquired resistance. The inclusion of Penicillin G in the susceptibility testing panel was primarily epidemiological and comparative, enabling confirmation of intrinsic resistance profiles and serving as a phenotypic control marker in antimicrobial resistance surveillance studies (CLSI, 2023; EUCAST, 2024). Furthermore, the highest sensitivity percentages were observed, namely 100% with gentamicin and azithromycin against *Salmonella typhi* isolates in the dry season and 100% with cefotaxime against *Salmonella typhi* isolates, as well as 100% with *Salmonella typhi*, *Shigella dysenteriae* and *Proteus vulgaris* isolates during the rainy season. During both seasons, azithromycin was the antibiotic with the highest sensitivity percentage for the different species isolated. During both seasons, azithromycin showed the highest susceptibility rates among the antibiotics tested against the different isolated bacterial species. This sustained activity may be explained by the relatively lower selective pressure exerted on macrolides compared with commonly used antibiotics such as β -lactams and tetracyclines, which are widely prescribed and often misused in many low- and middle-income countries (WHO, 2023; O'Neill, 2016). Moreover, azithromycin inhibits bacterial protein synthesis by targeting the 50S ribosomal subunit, a mechanism that remains comparatively less affected by the resistance mechanisms frequently encountered in environmental and enteric bacteria, such as β -lactamase production and efflux pumps (Roberts, 2008; Leclercq, 2002).

The stability of susceptibility profiles across seasons suggests that the antibiotic pressure on these ecosystems may not vary sufficiently to drive seasonal fluctuations in resistance to azithromycin, in contrast to what is often observed for other antibiotics intensively used during periods of high infectious disease incidence (Berendonk et al., 2015). However, increasing use of azithromycin in empirical treatments, particularly for gastrointestinal and respiratory infections, has been associated with the emergence of macrolide-resistant strains in several regions worldwide (Leclercq, 2002; WHO, 2023). Therefore, despite its current high efficacy, the present findings highlight the need for continuous antimicrobial resistance surveillance to prevent the rapid loss of this therapeutic option.

In terms of resistance rates to different families of antibiotics, multi-resistance was observed in Enterobacteriaceae species isolated from wastewater. The high resistance frequency values reflect the phenomenon of multi-resistance through the increase in resistant bacteria in the environment, which constitutes a major global health challenge (Hayward et al., 2020). Plasma resistance, which is the most common mechanism in Enterobacteriaceae, and chromosomal mutation could explain the observed resistance of bacterial species to certain antibiotics used.

The average wastewater temperature recorded at the various points is $25.75^{\circ}\text{C} \pm 1.65^{\circ}\text{C}$, which is below the Cameroonian standard NC 207 of 30°C . Some studies

showed that the temperature of wastewater in the city of Yaoundé meets discharge standards (Mbog, 2013). The pH of water represents its acidity or alkalinity and is related to the nature of the terrain it flows through (Derfouli et al., 2019). Overall, the pH of the water analysed fluctuates around $7.74 \text{ UC} \pm 0.51 \text{ UC}$. However, it was found that almost all wastewater sampling points had a slightly alkaline pH. The values obtained would be ideal for the growth of Enterobacteriaceae because a pH between 6 and 8.5 is favourable for the expression of the biological potential of several groups of bacteria. The average electrical conductivity value in wastewater ($1190.6 \mu\text{S/cm} \pm 644.04 \mu\text{S/cm}$) was higher than the Cameroonian standard of $1000 \mu\text{S/cm}$. In fact, an average value of electrical conductivity between 449.7 and $1037.3 \mu\text{S/cm}$ indicates high mineralization of wastewater and, therefore, for our various water samples, this could be due to high pollution from various anthropogenic activities (Belghyti et al., 2009). The average TDS value in wastewater ($594.1 \text{ mg/L} \pm 321.6 \text{ mg/L}$) was well above the standard (100 mg/L) and therefore constitutes a factor of water pollution as it is an indicator of aesthetic characteristics and the presence of a wide range of chemical contaminants (Atekwanaa et al., 2004). This result could be justified by the nature of the discharges that these waters receive, which are generally loaded with waste from anthropogenic activities (Taffouo et al., 2017). In wastewater, the average nitrate content obtained was $45.05 \text{ mg/L} \pm 53.2 \text{ mg/L}$, which is above the standard (15 mg/L) and can be explained by the presence of high levels of organic matter from the discharges it receives (Matini et al., 2009). It should be noted that the high nitrate levels obtained during the dry season are justified by the concentration of organic matter in the environment. The average value obtained was $4475.07 \text{ mg/L} \pm 3036.55 \text{ mg/L}$ of PO_4^{3-} , which is very high compared to NC 207 (2 mg/L of PO_4^{3-}). Like nitrates, the high concentrations of orthophosphate, which are indicators of organic pollution, can only be explained by the presence of very high organic loads from either domestic and/or hospital waste or anthropogenic activities around these biotopes (Matini et al., 2009). The high average nitrite content of $40.09 \text{ mg/L} \pm 89.77 \text{ mg/L}$ could be explained by the presence of large quantities of organic matter. These waters receive domestic waste rich in biodegradable organic matter directly, which increases the organic pollutant load. The average values observed for suspended solids ($579 \text{ mg/L} \pm 1139.57 \text{ mg/L}$) and colour ($3100.2 \text{ Pt. Co} \pm 4138.7 \text{ Pt. Co}$) are well above the drinking water quality standards of 5 mg/L and 15 Pt. Co . This can be explained by the organic pollution associated with the poor condition of the water pipes (lack of an effective sewerage system) and high levels of anthropogenic pollution. The results of the correlations between abiotic and biological variables show that, among the physicochemical parameters analysed, certain variables significantly influenced the population and distribution of bacteria throughout the study. According to Spearman's r correlation test, parameters such as TDS influence the growth of the different isolated species. Similarly, suspended solids, turbidity, and total dissolved solids were significantly correlated with the species *Shigella dysenteria*. These factors are believed to be elements that maintain

certain bacteria in the aquatic environment.

These significant correlations, obtained using Spearman's r correlation test, were confirmed by principal component analysis (PCA), which also showed that certain physicochemical parameters and Enterobacteriaceae species are either positively or negatively correlated with each other, while others show no significant correlation.

5. Conclusion

Our work shows that the wastewater analysed contains Enterobacteriaceae, the density of some of which is influenced by certain abiotic parameters. The diversity of Enterobacteriaceae species found in these aquatic biotopes includes *Salmonella typhi*, *Proteus vulgaris*, and *Shigella dysenteria*. The presence of these pathogenic germs shows that the waters analysed are polluted and carry these germs into the environment without treatment. Physicochemical analyses revealed that the analysed waters are alkaline, highly mineralised with little variation in temperature, and rich in organic matter and chemicals. In addition, the antibiogram performed with isolates of *Salmonella typhi*, *Shigella dysenteria* and *Proteus vulgaris* showed a multitude of multi-resistant strains varying during the seasons (dry or rainy). Significant correlations were noted between physico-chemical parameters and the abundance of enterobacteria such as *Shigella dysenteria*, confirming the influence of abiotic factors on bacterial growth.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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