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(RESEARCH ARTICLE)

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Sensitivity to antibiotics of bacteria of the genus *Aeromonas* isolated from developed groundwater points and some surface water points in the Commune of Nkolafamba (Centre Cameroon)

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Abstract

A study aimed at studying the sensitivity of bacteria of the *Aeromonas genus* to six (06) common antibiotics was carried out in groundwater and surface water points in the city of Nkolafamba (Centre Cameroon). The parameters considered were the physicochemical and bacteriological parameters. The water samples were taken and then analyzed using standard methods. The physicochemical parameters considered were the temperature, the pH among others. On the bacteriological level, in addition to the abundance dynamics of Heterotrophic Aerobic Mesophilic Bacteria (BHAM), that of bacteria of the genus *Aeromonas* as well as their sensitivity to antibiotics were monitored. The variables obtained were analyzed using the appropriate software.

Overall, it appears that the water temperature was closely related to the ambient temperature ($P<0.01$), the pH of the water is mostly acidic. This acidity was a function of the leached soils. The abundance of BHAMs varied over the study period from 10^4 CFU/100 ml to 2.5 x 10^6 CFU/100 ml. A total of 5 species of *Aeromonas* have been isolated : *A. media, A. jandaei, A. hydrophyla, A. schubertii* and *A. salmonicida.* All species usually present resistance to antibiotics. However, when the sensitivity was preserved, the average diameters of inhibition could reach 24 mm with gentamicin and 25 mm with ciprofloxacin for the species isolated from surface waters. The same inhibition diameters did not exceed 19 mm for groundwater. The resistance of bacteria to the usual antibiotics testifies to the need to carry out antibiogram tests before any health prescription in the event of contamination.

Keywords: Aeromonas; Antibiotics; Resistance; Sensitivity; Nkolamfamba

1. Introduction

The water consumed every day is essential to life [1] 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 [1]. 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 [2].

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The control of these waterborne diseases requires authorities to adopt new approaches that combine the control of cancer risks due to disinfection by-products with the control of the most resistant microorganisms [3,4]. The proposed objective is the absence of microorganisms in drinking water. This objective cannot be controlled by the usual indicators and an equivalent level of treatment is therefore recommended. The quality of the water is then controlled in real time by analyzing physicochemical parameters such as turbidity or measuring particles, and a posteriori control by new indicators such as spores of aerobic sporulating bacteria and those witnesses of faecal contamination. [5,6,7].

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 [8,9]. Thus, thin soil layers and high water tables contribute to groundwater vulnerability [8,10]. Both bacteria and pathogenic parasites originate from animal and human faeces [11]. 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 [5,6]. 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 [7,12]. 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%) [13, 14]. 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 [15,16].

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 of the *Aeromonas genus* in borehole and surface water used for drinking water and sensitivity of these germs to antibiotics from bacteria of the genus *Aeromonas* isolated from well water and surface water. The present work aims to evaluate the antibiotic sensitivity of bacteria of the genus *Aeromonas* isolated from underground water points and in some surface water points in the Commune of Nkolafamba (Centre Cameroon).

2. Material and methods

2.1. Sample collection _

2.1.1. Choice of sampling points

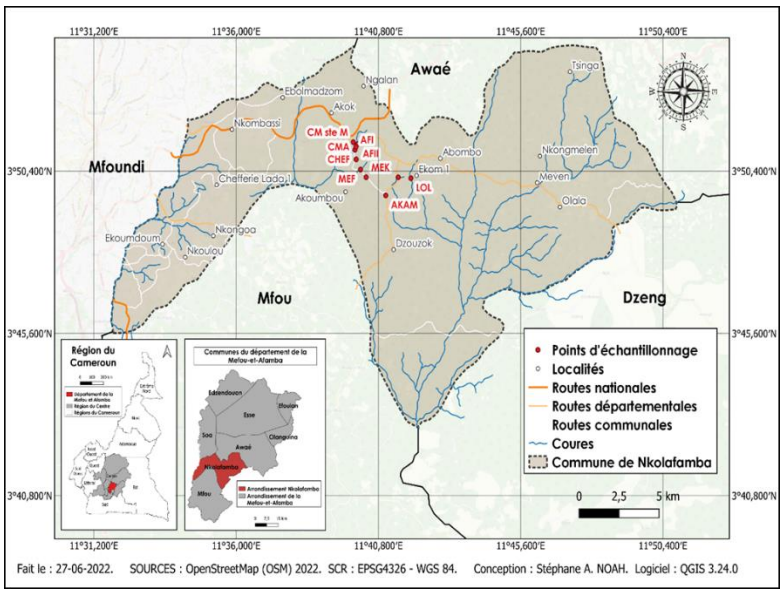

Figure 1 Positions of sampling points in the Arrondissement of Nkolafamba (Mairie de Nkolafamba, 2022)

Ten (10) water points have summer chosen namely five surface and five underground in different districts of the city on the basis of criteria such as the accessibility of the points, the interest that the populations show for these water

points, the presence of one possible source of pollution and in order to best cover the whole area. He was considered than a water point East all the more important as the volume of water drawn East high and / or water drawn East in priority intended for food human. Table 1 summarizes the water point codes underground analyzed, their contact details geographic locations and their average altitudes. These contact details geographic locations and the altitude of the various boreholes summer obtained in the field using a GPS map. The ten (10) sampling points are coded. Campaigns _ sampling have summer carried out from January to July 2021 following a monthly step sampling. The water has been taken at each point in using different containers prepared in the laboratory for this effect. Figure 1 shows the location of these points on the map of the city of Nkolafamba.

2.2. Physicochemical parameters analyzed

The physicochemical parameters were analyzed using the Techniques developed by [17]. Table 2 summarizes the parameters considered, the technique, the measurements and units of measurements.

Table 1 Geographical coordinates of sampling stations and point pollution points

Stations	Codes	Altitudes (m)	Point pollution point	security fence	Geographical locations
Ste Marie Medical Office	CM ste M	696	Toilet	Absent	3°51'15.623''N 11°39'56.95''E
CMA of Nkolafamba	CMA	609	Garbage can	Absent	3°51'7.075''N 11°40'2.460''E
Mekon	MEK	720	-	Present	3°50'13.29''N 11°40'23.338''E
Chiefdom	CHIEF	686	-	Absent	3°50'44.79''N 11°40'2.96''E
A kam	AKAM	717	Crop (fertilizer)	Present	3°49'55.82''N 11°37'16.9''E
Otoundoumba	OTTOU	719	Detergent	-	03°53'11.1''N 11°50'28.5''E
Lolo	LOLO	699	Detergent	-	03°59'16.4''N 11°43'20.2''E
Afamba 1	AFI	670	Detergent and fertilizer	-	03°52 ' 34''N 11°37'16.9''E
Afamba 2	AFII	667	Detergent and fertilizer	-	03°52'12.3''N 11°40'21.9''E
Mefomo	MEF	698	Detergent	-	03°43 ' 25.7''N 11°40'33.3''E

Table 2 Parameters analyzed, methods of measurement, devices and units used for each parameter [17]

Parameters	Technical	Site	Apparatus	Units
Temperature	Direct	In situ	thermometer	°C
Ph	Direct	In situ	pH-meter	CU
Conductivity	Direct	In situ	conductimeter	$\mu S.cm^{-1}$
Dissolved O_2	Volumetry by $Na_2S_2O_3$	Laboratory	Titrimetry	% saturation
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	$mg\ l^{-1}$
SuspendedMatter	Colorimetry (810nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
Color	Colorimetry (455nm)	Laboratory	Spectrophotometer	Pt.Co
PO_4^{3-}	Colorimetry (880 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
NO_3^-	Colorimetry (570 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
$NH4^+$ _	Colorimetry by Nessler (425nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$

2.3. Sample analysis

Samples _ water intended for bacteriological analyzes have summer collected in vials 500 ml glass previously sterilized. For physicochemical analyses, samples have summer collected in vials 1000 ml double capped polyethylene [17]. All these samples have summer transported to the laboratory in a refrigerated enclosure or they have summer immediately analyzed.

2.3.1. Choice of germs

Germs _ research have been the BHAMs, the bacteria pathogens of the genus *Aeromonas.* The BHAMs have summer wanted to have an idea about flora total mesophilic viable [11], bacteria pathogens *Aeromonas.* have summer chosen due to their recurrent involvement in waterborne diseases and epidemics in developing countries emergence [18].

2.4. Germ isolation _

Germ isolation was _ _ performed by the spreading technique on the surface for the BHAMs. 100 µL of sample have summer collected using _ a tensor pipette sterile Then deposited on the surface of ordinary agar The sample was then spread using a spreader _ in glass sterile Petri dishes have Next summer incubated at temperature ambient for 1 to 5 days [11]. With regard to bacteria of the *Aeromonas* genera, the isolation was done by the membrane filter technique with cellulose ester membranes Millipore, Bedford, MA 01730 with a porosity of 0.45 µm [19]. 100 mL of water sample were filtered through a sterile squared filtering membrane, using fine tweezers previously passed through the flame of the bunsen burner, the membrane was then placed very gently in the petris dishes containing the ADA culture media poured into 55 mm Petri dishes and incubated at 37 °C. for 24 ± 3 hours. [11]. The manipulations have summer made in a diameter of 30 cm around the flame of the spout Bunsen [19].

2.5. Determination of abundances bacterial

For each campaign sampling, bacteria isolated have summer counted by direct counting using a pointer colony counter) this shows bacterial colonies of shapes, colors, sizes and appearance varied. [20]. The concentrations have summer expressed in Colony Forming Unit per 100 ml (CFU/100 mL).

2.6. Identification of germs

For the identification of bacteria belonging to the genera *Aeromonas,* after gram staining, basic tests have summer made using the gallery _ classic. We can mention the search for oxidase, the search for the enzyme catalase, the fermentation of glucose, the production of gas, the affinity for oxygen (aerobic facultative anaerobe), motility, mannitol fermentation, lactose fermentation, H_2S production, urease research, citrate utilization among others [11,19]. The identifications were carried out on the *Aeromonas genera* with the aim of determining the species corresponding to the colonies thus isolated on Petri dishes and presenting satisfactory cultural characteristics [20]**.**

2.7. Assessment of antibiotic susceptibility

2.7.1. Preparation of the inoculum

From a pure and young culture of 18 to 24 hours on specific medium, a bacterial suspension in saline solution 9% of turbidity equivalent to that of standard 0.5 of the range of Mac Farland was carried out.

2.7.2. Choice of antibiotics and performance of antibiogram tests

The antibiogram tests were carried out on the presumptive strains at the end of the identification tests by the method of diffusion of discs impregnated with antibiotics on Müller Hinton agar medium according to the recommendations of the Antibiogram Committee of the French Society of Microbiology. For this purpose the discs containing the antibiotics cephalosporins, penicillin, fluoroquinolones among others (Table 3) were chosen. The results were recorded as resistant or susceptible by measuring the diameter of the zone of inhibition using the caliper, according to the interpretative level of the [21]. The values of the critical reference diameters for each antibiotic which make it possible to determine sensitivity, resistance or intermediateness are recorded in Table 3 [21].

2.8. Seeding and depositing discs _ antibiotics

During the study, antibiogram tests were carried out during the long dry season and the short rainy season for all ten (10) sampling sites (5 wells and 5 surface water points) and on these different points it was tested each strain of *Aeromonas* to six (6) different antibiotics.

The bacterial suspensions were inoculated using the swabs on the entire surface of the ADA agar 4 mm thick. The inoculated dishes were left to dry for 15 minutes in the open air near the flame before depositing the antibiotic discs (maximum six discs per dish of 90 mm in diameter). One disk was placed in the center and the others were placed 15 mm from the edge of the box. The gap between the discs is 30 mm. Petri dishes were incubated aerobically at 35 ± 2°C for 16 to 24 hours. After incubation, the reading was done by measuring the diameters of the zones of inhibition using a caliper and by referring to CA-SFM standards to declare the germ sensitive, intermediate or resistant compared to the critical diameters of the antibiotic tested [22].

Table 3 List of antibiotics used and critical reference diameters of the various antibiotics used

Classes of antibiotics	***Aeromonas***	
	Antibiotics	**Disc loads (µg)**
Cephalosporins	Cefotaxim	30
Fluoroquinolones	Ofloxacin	5
	Ciprofloxacin	5
Penicillin	Ampicillin	10
	Amoxicillin	30
Various	Trimethoprim-sulfamethoxazole	25
	Chloramphenicol	30
Cephalosporins	Ceftriaxone	30

Reference critical diameters for each Antibiotic						
Antibiotics	NC	VS	CMA	AX	C.I.P.	KFO
Sensitive	≥18	≥27	≥23	≥ 24	≥25	≥ 26
Intermediate	12-18	21-27	17-23	18-24	19-25	18-26
Wearing	< 12	<21	<17	<18	< 19	<26

Legend: CN: gentamicin; C: chlormphenicol; AMC: amoxicillin+acid clavinic; AX: amoxicillin; CIP: ciprofloxacin; KFO: kanamicin

2.9. Data analysis

2.9.1. Spearman rank correlation coefficient

The Spearman rank correlation coefficient was determined from SPSS 20.0 software. This coefficient made it possible to establish the correlations between the biological and abiotic variables.

2.9.2. Comparisons

The comparisons between the variables considered were carried out using the Kruskal-Wallis "H" comparison tests and the Mann-Whitney "U" tests using the PAST software.

2.9.3. PCA (Principal Component Analysis)

In this study, a PCA was carried out in order to characterize the sampling stations on the basis of the bacterial concentrations in relation to the physicochemical parameters. The objective of this descriptive analysis method is to present in the form of a graph, the maximum of the information contained in a large data table.

3. Results

3.1. Settings physicochemical

3.1.1. Physical Parameters

The physical parameters during the samples were measured by appropriate techniques. It has been observed that these parameters vary overall from station to station and from sample point to sample point (Figure 2).

The measured temperature values fluctuated between 22 and 31.2°C. The lowest value was recorded at Mekon and Mefomo stations in mid-July and Afamba 1 and 2 in August. And the highest value at the CMA station in June with an average value of 24.923 ± 2.05 °C. The highest turbidity value (33 FTU) was recorded in early June at the Lolo station. The lowest value (0 FTU) was recorded at the end of June at the CMA, Akam and Mekon stations with an average value of 6.05 ± 7.755 FTU. Overall, the water color values oscillated between 2 and 206 Pt.Co with an average value of 45.083 ± 54.583 Pt.Co.

3.1.2. Chemical parameters

Overall, physicochemical parameters varied from one sampling station to another and from one sampling period to another (Figure 6). Regarding dissolved oxygen, the lowest value was recorded in the Chefferie and Akam stations in August and the highest value was observed in June at the Otoundoumba station. (Figure 3A).

The pH values of the water during sampling varied from 5.58 to 7.08 UC. The maximum value was recorded in early June at the LOLO station and the minimum value in mid-July at the Chefferie station. With an average value of 6.408 ± 0.433UC (FIG. 3B). Spatially, the Kruskal -Wallis H test shows that there is a significant difference between the different stations ($p < 0.05$).

The electrical conductivity values fluctuated between 12 and 466 µs/cm. The highest value was recorded at Akam station in July. The lowest value at the Chefferie station in August (Figure 3C).

The nitrate content is higher in June at the Center station (169 mg/L). The lowest (1 mg/L) in the Afamba 2 station in July and in August in the Afamba 1 and CMA stations (figure 3D).

The orthophosphate water content reached 4.12mg/L in August at the Center station. They were rare at the end of July in the Mekon, Chefferie and Center stations (Figure 3E). Spatially, the Kruskal -Wallis H test shows that there is no significant difference between the different stations ($p > 0.05$).

Overall, dissolved $CO_{2\ values}$ fluctuated between 1 and 30.84 mg/L. The highest value was recorded at the Mekon station at the end of August. The lowest value was obtained at the Afamba 1 station in June. However, there is an average value of 7.286 ± 6.06mg /L (Figure 3F). Spatially, the Kruskal -Wallis H test shows that there is no significant difference between the different stations ($p > 0.05$).

During the study period, the ammonia nitrogen contents fluctuate between 0.00 and 16.70 mg/l with an average of 1.71±3.34mg/l and an amplitude of 16.70 mg/l. high levels are observed in mid-July at all stations (Figure 3G). On the spatial level, the Kruskal Wallis test shows that there is no significant difference between the different stations ($p > 0.05$).

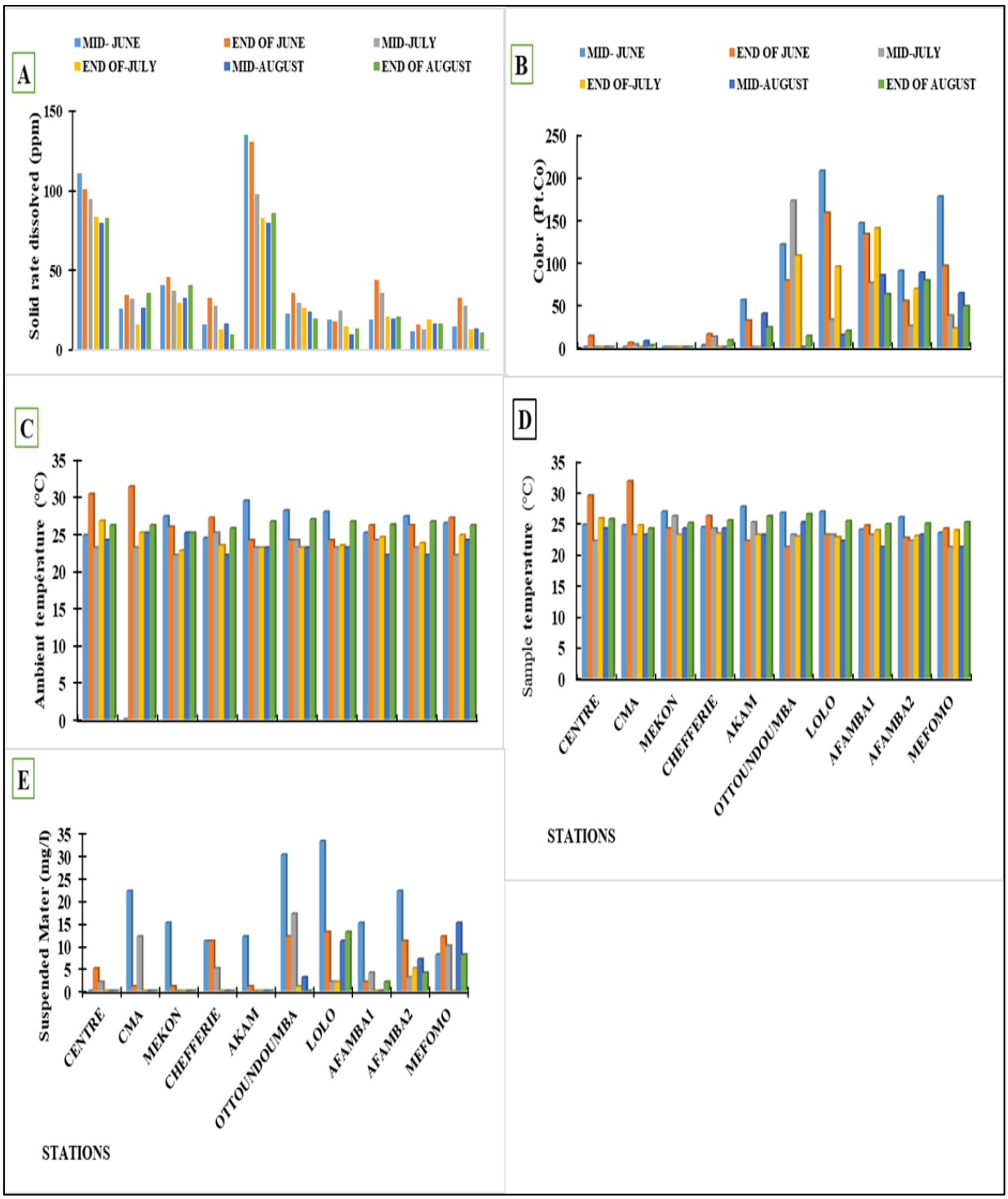

Figure 2 Spatiotemporal variations of physical parameters (A: TDS; B: Color; C: Ambient temperature; D: Water temperature; E: MES)

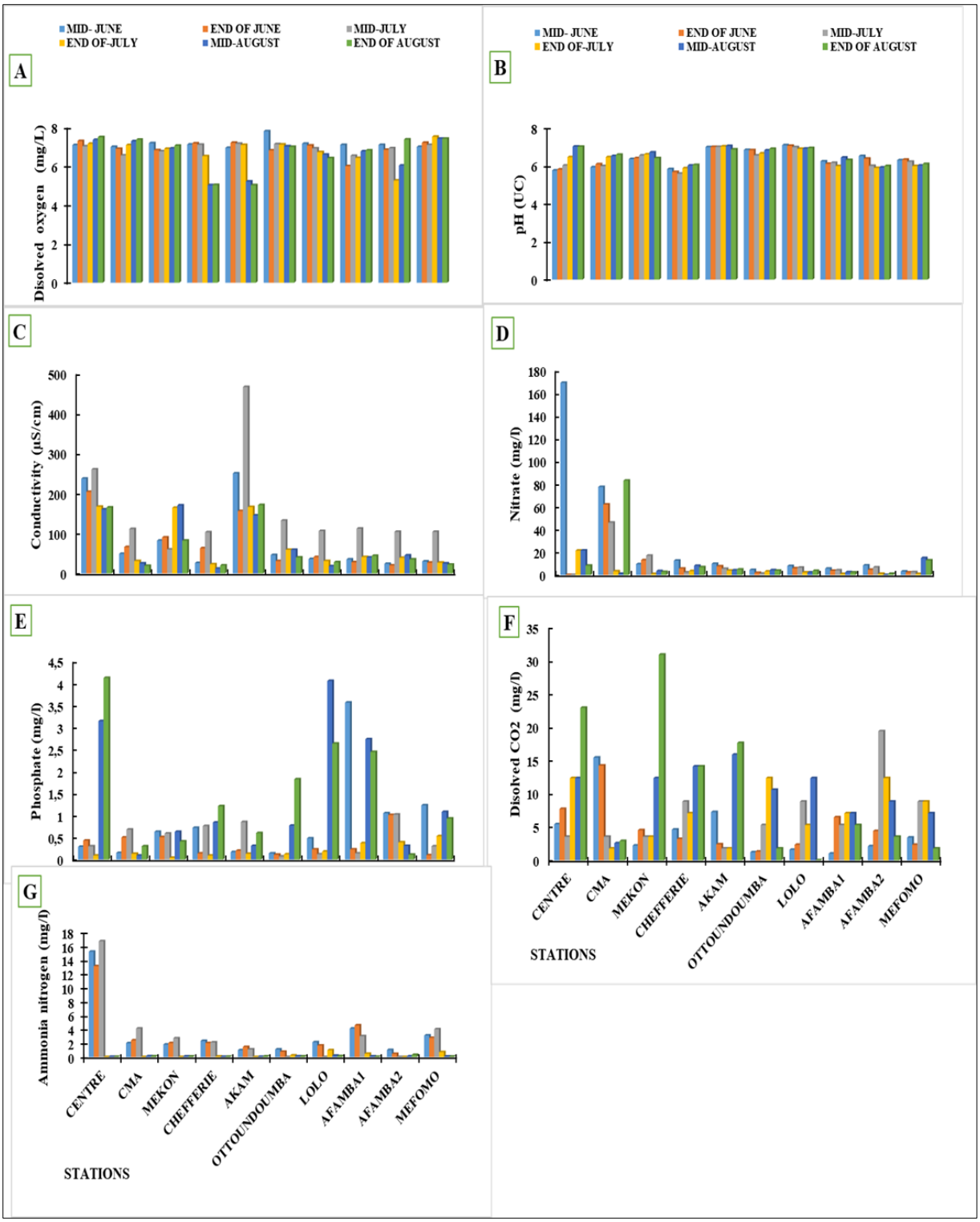

Figure 3 Spatiotemporal variations of chemical parameters A: O2; B: pH; C: Conductivity; D: Nitrate; E: Orthophosphate; F: CO_2; G: Ammonium)

3.2. Biological variable

3.2.1. Qualitative aspect

Germ isolation

After growth on ampicillin and dextrin agar, after macroscopic observation, the colonies which have brought attention are those having satisfactory cultural characteristics to those of *Aeromonas.* These different cultural characteristics are as follows :

- Milky white colonies 1-2 mm in diameter (A) ;
- Milky white colonies with compact center with blue halo 2-3 mm in diameter (B) ;
- Milky white colonies with a 3 mm yellow center forming the blue halo (C) ;
- Milky white colonies with a yellow center 2 mm in diameter (D) ;
- Milky white colonies 3 mm in diameter (E)

Identification of isolated germs

Table IV presents the results of the various identification tests carried out on the isolated bacterial strains. Indeed, the tests carried out from the colonies isolated on the ADA medium made it possible to identify five (05) species of *Aeromonas* qualified as opportunistic pathogens which are: *A. media, A. jandaei, A. hydrophyla, A. schubertii* and *A. salmonicida.* It was observed that all these colonies are able to synthesize catalase. They are thus able to live in the presence of oxygen. In addition, all the strains isolated do not ferment lactose (Table 4).

Table 4 Results of the identification tests carried out

Identification tests	**Bacterial strains**				
	A	**B**	**C**	**D**	**E**
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Mobility	-	+	+	+	+
Urease	-	-	-	-	-
H2S	-	+	+	-	+
Citrate	-	+	-	-	+
ADD	-	-	-	-	-
Indole	+	+	+	+	+
Gas	+	+	-	-	+
Glucose	+	+	-	-	+
Lactose	-	-	-	-	-
D-Mannitol	+	+	-	+	+
DHA	-	-	+	-	+
CDL	-	+	+	+	+
ODC	-	-	-	-	-
Species identified	***A.media***	***A. jandaei***	***A. shubertii***	***A. salmonicida***	***A. hydrophila***

Legend: +: Ferments the substrate or synthesizes the enzyme/gas; -: does not use the substrate or produce the enzyme/gas; A, B, C, and D represent the cultural characters of the colonies observed in isolation; ADH: Arginine Di Hydrolase; LDC: Lysine Decarboxylase; H2S: Dihydrogen sulfide; ODC: Ornithine Decarboxylase; TDA: Tryptophan deaminase.

3.2.2. Quantitative aspect

Variations in bacterial abundances

The abundances of isolated cells varied overall from one sampling point to another, from one sampling period to another and from one species considered to another (Figure 4).

Overall, BHAM densities fluctuated between 10^4 CFU/100mL and $2.5x10^6$ CFU/100mL of water. The lowest abundance was obtained at the Chefferie station in August and the highest abundance at the Mefomo station during the month of June. Spatially, the Kruskal -Wallis H test shows that there is no significant difference between the different stations ($p > 0.05$).

As for *A. hydrophyla,* their density varied from 1 to 1440 CFU/100mL of water. The smallest value was recorded at the end of July in the Mekon and Otoundoumba stations and the highest value was recorded at the same period in the CMA station. The density of *A. media* reached its highest value in July 1366 CFU/100 ml of water at the Afamba 1 station and the smallest value this same month at the Mékon station (3 CFU/100 ml of water). The presence of *A. jadaei* and *A. schubertii* was sometimes rare in almost all the stations except at the Lolo station in July where we observe their highest values 900 CFU/100 ml of water for *A. jadaei* and 1070 CFU/ 100 ml of water for *A. schubertii.*

Concerning *A. Salmonicida,* its lowest value was observed in June at the Center station and the highest value in June and August 1335 CFU/100 ml of water in the Ottou and Afamba 1 stations Spatially, the Kruskal-Wallis H test shows that there is no significant difference between the different stations ($p > 0.05$).

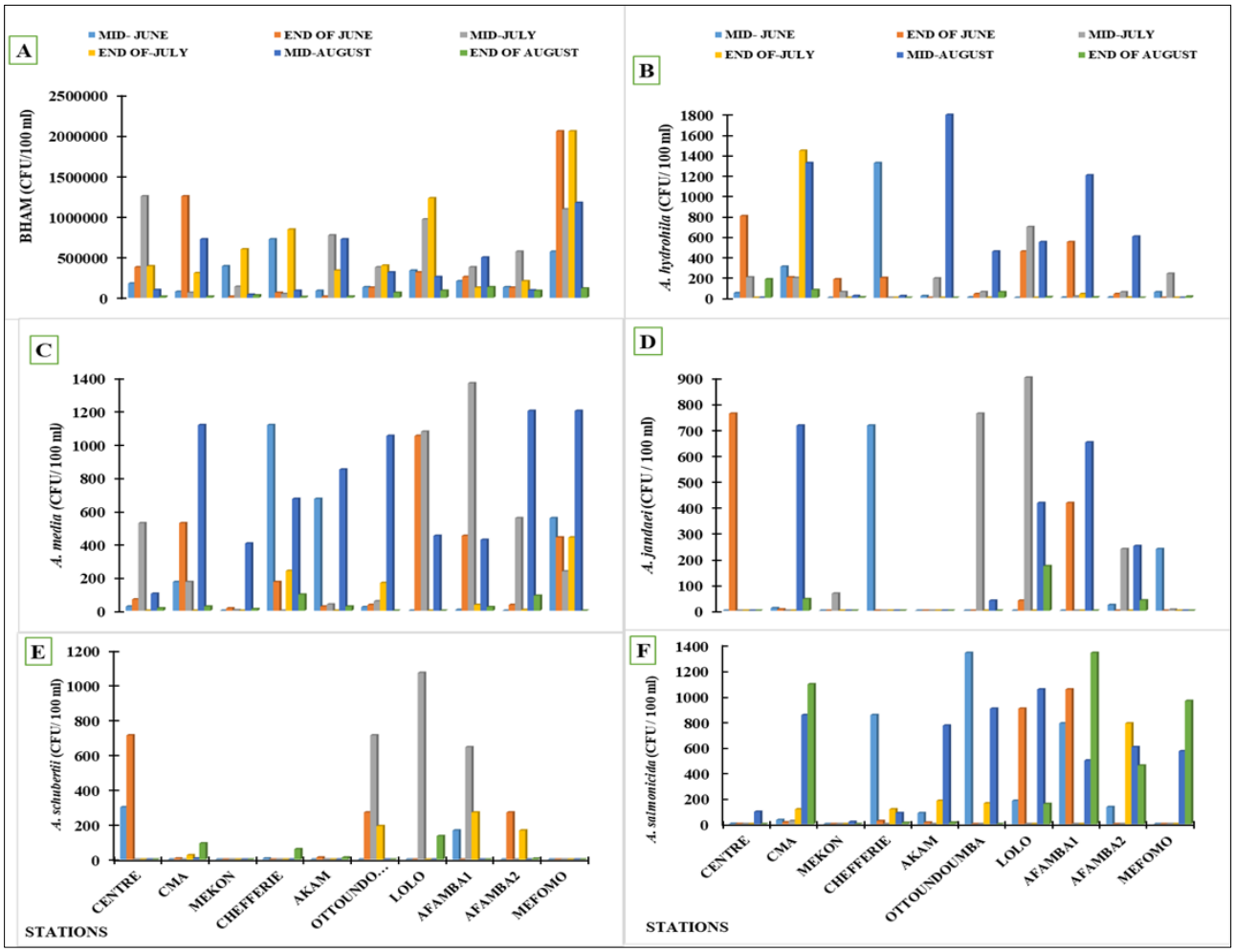

Figure 4 Spatiotemporal variations in bacterial abundances (A: BHAM; B: *A. hydrophila;* C: *A. media;* D: *A. Jandeii;* E: *A. schubertii;* F: *A. salmonicida)*

3.3. Antibiotic sensitivity

3.3.1. Antibiogram performed

In short, six (06) antibiotics commonly used by populations and belonging to the families of Beta lactams, Phenicoles, Quinolones and Aminosides were used to carry out the antibiogram tests on the identified strains. These are gentamicin, ciprofloxacin, chlormphenicol, amoxicillin plus clavulanic acid, amoxicillin, and kanamicin. Inhibitions have been observed overall, the diameters of which on macroscopic observation vary according to the antibiotic and the species considered respectively.

3.3.2. Antibiotic sensitivities of strains isolated from groundwater sources

The sensitivity of the isolated bacteria to antibiotics was considered by measuring, using a caliper, the diameters of inhibitions around the discs soaked in antibiotic of the cells previously cultured on Muller-Hinton medium. The means of these inhibition diameters are presented in Table 5.

Overall, we note that these averages vary between 5 mm and 19 mm in the well water (groundwater) used mainly for drinking water. The lowest mean inhibition diameters (5 mm) were observed for amoxicillin and kanamicin at all sampling points, whereas the highest inhibition diameter (19 mm) was observed at the point of Akam water. According to the standards of the French Society of Antibiogram (2020), resistance to antibiotics is observed when the diameter of inhibition is lower than the standard, the resistance is intermediate if the diameter is included in the standard and the bacterium is susceptible to the antibiotic if the inhibition diameter is greater than the norm margin. Thus, a sensitivity of *Aeromonas* isolated from borehole waters in downtown Nkolafamba and Akam to gentamicin has been observed. It was obtained an intermediate resistance of Aeromonas isolated from the waters of the boreholes of Mekon and the traditional Chiefdom of Nkolafamba to gentamicin on the one hand, and of the waters of the Akam borehole to ciprofloxacin on the other hand. Most of the time, *Aeromonas* are resistant regardless of the antibiotic considered or the water point where they were isolated (table 5).

Table 5 Mean values of inhibition diameters in mm of isolated *Aeromonas* (susceptibility to the antibiotic) in underground water points (drilling)

Antibiotic	Standard	CENTER	CMA	MEKON	HEADQUARTERS	AKAM
CN	(12-18)	18(S)	11 (R)	14 (I)	14 (I)	19(S)
C	(21-27)	17.7 (R)	18 (R)	8.3(R)	11 (R)	15 (R)
AMC	(17-23)	8.7(R)	14 (R)	17.3 (R)	8 (R)	10 (R)
AX	(18-24)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)
C.I.P.	(19-25)	14.3 (R)	15 (R)	11 (R)	18 (R)	19 (I)
KFO	(18-26)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)

Legend: CN: gentamicin; C: chlormphenicol; AMC: amoxicillin+acid clavinic; AX: amoxicillin; CIP: ciprofloxacin; KFO: kanamicin; I: Intermediate (intermediate resistance to the antibiotic); R: Resistant to antibiotic; S: Sensitive to antibiotic.

3.3.3. Antibiotic sensitivities of strains isolated from surface water sources

Table 6 Mean values of the inhibition diameters in mm of isolated *Aeromonas* (susceptibility to the antibiotic) in surface water points

Antibiotic	Standard	LOLO	AFAMBA 1	AFAMBA 2	OTTOUNDOUMBA	MEFOMO
CN	(12-18)	24(S)	14 (I)	6.5 (R)	19(S)	14 (I)
C	(21-27)	14.5 (R)	15.5 (R)	12.5(R)	19 (I)	18.5 (R)
AMC	(17-23)	14 (R)	13.5 (R)	9 (R)	12.3 (R)	10 (R)
AX	(18-24)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)
C.I.P.	(19-25)	5 (R)	4.5 (R)	19 (I)	21 (I)	25 (S)
KFO	(18-26)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)

Legend: CN: gentamicin; C: chlormphenicole; AMC: amoxicillin+acid clavinic; AX: amoxicillin; CIP: ciprofloxacin; KFO: kanamicin; I: Intermediate (intermediate resistance to the antibiotic); R: Resistant to antibiotic; S: Sensitive to antibiotic

In the surface waters of the city of Nkolafamba, the results of the analysis of the sensitivity to antibiotics of isolated *Aeromonas* show inhibition diameters which vary between 5 mm and 24 mm when all the points sampled are considered and all of the antibiotics tested (Table 6). A sensitivity to gentamicin was noted in *Aeromonas isolated from Lolo and* Otoundoumba water points, and to ciprofloxacin in cells isolated from the Mefomo water point. Additionally, *Aeromonas isolated from* Afamba 1, Afamba 2, Otoundoumba, and Mefomo sampling points may show intermediate resistance to antibiotics such as gentamicin, chloramphenicol, and ciprofloxacin. Most of the time, resistance was observed regardless of the antibiotic considered or the water point sampled (Table 6).

3.3.4. Behavior of each isolated bacterial species against antibiotics

The behavior of the isolated species against antibiotics at each sampling point was analyzed (Table 7). This exercise was carried out in order to verify the effective antibiotic that acts on the isolated cells in order to guide the health authorities for prescriptions in the event of infection. The germs tested are the most abundant. It appears that most of the time, all the species identified are resistant to the antibiotics considered, whatever the sampling point. However, intermediate and sensitive reactions of *Aeromonas* on antibiotics can be observed can be observed (Table 7).

Table 7 Mean values of the inhibition diameters in mm of the *Aeromonas* species isolated (and sensitivity to the antibiotic) in the sampling water points

	C	**CN**	**AMC**	**KFO**	**C.I.P.**	**AX**
A. salmonicida (Ottou)	14 (R)	16 (I)	13 (R)	0 (R)	22 (I)	0 (R)
A. hydrophila (Ottou)	13 (R)	16 (I)	12 (R)	0 (R)	16 (R)	9 (R)
A. schubertii (Ottou)	20 (R)	20 (I)	12 (R)	0 (R)	43(S)	0 (R)
A. hydrophila (AF1)	20 (R)	14 (R)	15 (R)	0 (R)	15 (R)	15 (R)
A. jandaei *(* AF1 *)*	22 (I)	17 (I)	14 (R)	0 (R)	25 (I)	0 (R)
A. hydrophila (CHIEF)	8 (R)	13 (I)	1 (R)	0 (R)	14 (R)	0 (R)
A. media (CHIEF)	22 (I)	13 (I)	1 (R)	0 (R)	14 (R)	0 (R)
A. schubertii (CMA)	15 (R)	15 (I)	1 (R)	0 (R)	17(R)	0 (R)
A. jandaei (MEF)	21(S)	1 (R)	8 (R)	0 (R)	15 (R)	0 (R)
A. media (AKAM)	14 (R)	15 (I)	11 (R)	0 (R)	13 (R)	/
A. schubertii (MEK)	29 (S)	1 (R)	13 (R)	0 (R)	13 (R)	10 (R)
A. schubertii (LOLO)	22 (I)	16 (I)	10 (R)	/	35 (S)	0 (R)
A. media (AF2)	20 (I)	16 (I)	10 (R)	0 (R)	0 (R)	0 (R)
A. jandaei (AF2)	22 (I)	17 (I)	0 (R)	0 (R)	36(S)	0 (R)
A. salmonicida (AF2)	21 (I)	19(S)	13 (R)	0 (R)	42(S)	0 (R)
A. hydrophila (AF1)	17 (I)	12 (I)	14 (R)	0 (R)	21 (I)	1 (R)
A. salmonicida (LOLO)	22 (I)	18(S)	0 (R)	0 (R)	25 (S)	0 (R)

Legend: CN: gentamicin; C: chlormphenicole; AMC: amoxicillin+acid clavinic; AX: amoxicillin; CIP: ciprofloxacin; KFO: kanamicin; I: Intermediate (intermediate resistance to the antibiotic); R: Resistant to antibiotic; S: Sensitive to antibiotic; AF1: Afamba 1; AF2: Afamba 2; Ottou : Ottoundoumba ; /: reaction to the antibiotic not observed.

3.4. Correlations between the variables considered

3.4.1. Correlations between physicochemical variables

Overall, correlations between the abiotic water variables were obtained for all the water points considered (Table 8). These correlations are very significant and positive between the ambient temperature and that of the water sampled ($P<0.01$; $r=0.679$), between the color of the water and the SS content ($P<0.01$; $r=0.524$) and between the contents of NH_4^+ and SS ($r=0.397$) among others (Table 8). Conversely, very significant and negative correlations ($P<0.01$) were observed between the dissolved $CO_{2\ contents}$ and those of SS ($r=-0.349$) in the water.

Table I Spearman's "r" correlation coefficients between physicochemical parameters

Settings	O_2 dissolved	Ph	TDS	Condition	Color	NO_3^-	PO_4^{3-}	am T°.	Sampling T °	dissolved CO_2	MY	$NH4^+$
O_2 dissolved	1											
pH	0.059	1										
TDS	0.135	0.228	1									
Condition	0.078	0.192	0.779**	1								
Color	-0.036	0.049	-0.342*	-0.312**	1							
NO_3^-	0.137	0.116	0.164	0.062	-0.31	1						
PO_4^{3-}	-0.102	0.011	-0.292*	-0.187	-0.058	0.237	1					
am T°.	0.337**	0.01	0.08	-0.092	0.187	0.136	0.072	1				
Sampling T °	0.198	0.02	0.02	0.118	-0.233	0.36*	0.139	0.679**	1			
dissolved CO_2	-0.297*	-0.197	0.082	0.222	-0.235	0.024	0.121	-0.273*	0.078	1		
MY	0.172	-0.102	-0.274*	-0.207	0.524**	0.055	0.026	0.215	-0.008	-0.349**	1	
$NH4^+$	0.098	-0.286*	0.217	0.051	0.178	0.081	0.01	0.192	0.132	-0.302*	0.397**	1

Legend: Cond: Conductivity; am T°.: Ambient temperature; Sampling T °: Sampled water temperature; N=60; *: Significant (P<0.05); **: Very significant (P<0.001)

3.4.2. Correlations between bacteriological variables

The analysis of the correlations between the abundances of isolated bacteria show that the increase in *A. media densities* is very significantly concomitant with the increase in BHAM, *A. hydrophila and A. jandaei densities.* With correlation coefficients of 0.33, 0.486 and 0.386 respectively (P<0.01). The same result was observed between the abundances of *A. jandaei* and those of *A. hydrophila* (P<0.01; r=0.579) (Table 9).

Table 9 Spearman's "r" correlation coefficients between bacteriological parameters

Species	**BHAM**	***A. hydrophila***	***A. media***	***A. jandaei***	***A. shubertii***	***A. salmonicida***
BHAM	1					
A. hydrophila	0.101	1				
A. media	0.33**	0.486**	1			
A. jandaei	0.197	0.579**	0.386**	1		
A. shubertii	-0.052	0.068	0	0.164	1	
A. salmonicida	0.102	0.17	0.17	0.222	-0.058	1

Legend: N=60; *: significant (P<0.05); **: very significant (P<0.01)

3.5. Correlations between bacteriological and physicochemical variables

3.5.1. Correlations between bacterial densities and physicochemical variables

Phycicochemical variables and bacterial densities were performed using Spearman's "r" test. Overall, there are degrees of significance which differ according to the density of the species and the value of the phycicochemical parameter considered (Table 10). A very significant and negative correlation was noted between the levels of electrical conductivity of the water and the densities of *A. salmonicida* (P<0.01; r=-0.388) and between the temperature of the water sampled and the abundances of *A. media* (P<0.01; r=-0.345). Moreover, the results show significant and negative correlations (P<0.05) between the nitrate contents and the abundances of BHAMs (r=-0.261); between room temperature and the abandances of *A. hydrophila* (r=-0.262) and *A. media* (r=-0.312) respectively (Table 10). However, a significant and positive correlation was observed between dissolved CO_2 contents and *A. media* densities.

3.5.2. Correlations between bacterial inhibition diameters and physicochemical variables

Table 10 Spearman's "r" correlation coefficients between physicochemical parameters and bacterial densities

Settings	**BHAM**	***A. hydrophila***	***A.media***	***A. jandaei***	***A.schubertii***	***A. salmonicida***
O_2 dissolved	0.114	-0.167	-0.15	-0.028	-0.077	0.027
Ph	-0.039	-0.028	-0.189	-0.062	-0.106	0.058
TDS	-0.127	0.077	-0.071	-0.209	0.045	-0.277*
Conductivity	-0.017	0.056	-0.058	-0.186	-0.036	-0.388**
Color	0.156	-0.091	0.136	0.18	0.205	0.204
Nitrate	-0.261*	-0.018	-0.056	-0.109	-0.109	0.098
Phosphate	-0.248	0.04	-0.061	0.017	-0.216	0.104
Ambient temperature	-0.227	-0.262*	-0.312*	-0.129	0.109	0.03
Sampled water temperature	0.323*	0.099	-0.345**	0.075	0.132	-0.141
dissolved CO_2	-0.048	0.085	0.367**	0.058	-0.059	-0.119
SS	0.061	-0.013	0.062	0.196	0.006	0.184
Ammonium	0.099	0.143	0.132	0.083	0.013	-0.108

Legend: N=60; T°: Temperature; *: significant (P<0.05); **: very significant (P<0.01)

The analysis of the correlation tests carried out between the diameters of inhibition of the bacteria after the antibiogram and the abiotic variables show on the whole a very significant and positive correlation (P<0.01) between the dissolved O 2 contents (r=0.841), the ambient temperature (r=0.770) and the variation in the diameter of inhibition of *Aeromonas* on amoxicillin. A significant and negative correlation was observed between the contents of MES and the variation in the diameter of the bacteria on the gentamicin (Table 11).

Table 11 Spearman's "r" correlation coefficients between physicochemical parameters and bacterial inhibition diameters

Settings	CN	AMC	AX	C.I.P.	KFO
O 2 dissolved	-0.24	0.499	0.841**	0.451	-
Ph	0.5	-0.062	0.372	0.201	-
TDS	0.616	0.092	0.323	-0.091	-
Condition	0.571	-0.571	-0.068	0.299	-
Color	-0.519	0.178	-0.073	0.427	-
NO 3 -	0.389	-0.037	0.122	-0.11	-
PO43-	-0.117	0.492	-0.256	0.232	-
Ambient temperature	-0.062	0.13	0.77**	0.365	-
Sampling T °	0.558	0.08	0.116	-0.091	-
dissolved CO 2	0.532	-0.074	-0.128	-0.616	-
MY	-0.649*	0.012	0.122	0.378	-
NH4 + _	-0.519	0.178	0.116	-0.091	-

Legend: Cond: Conductivity; am T°. : Ambient temperature ; Sampling T ° : Sampled water temperature; N=10; *: Significant (P<0.05); **: Very significant (P<0.001); CN: gentamicin; C: chlormphenicole ; AMC: amoxicillin+acid clavinic ; AX: amoxicillin ; CIP: ciprofloxacin; KFO: kanamicin ; -: not observed.

3.6. Principal Component Analysis (PCA)

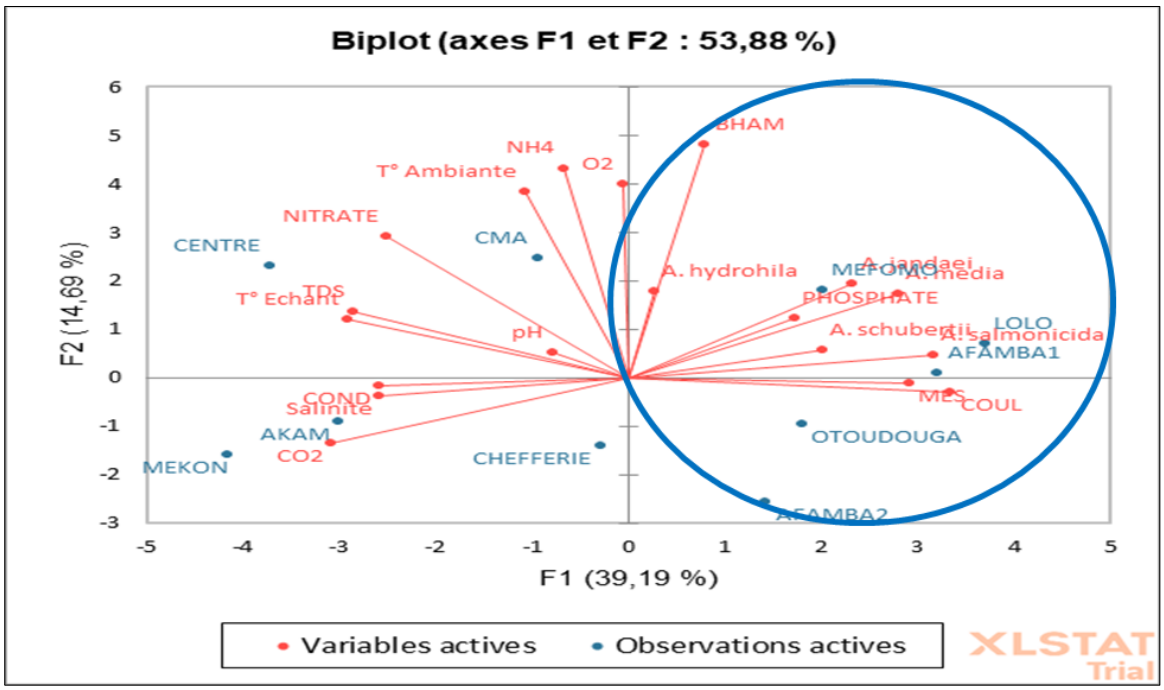

Figure 5 PCA values grouping the affinities between the bacterial abundances and the physicochemical and hydrological parameters

The PCA applied to the different bacteriological densities and to the physicochemical variables for all the sampling points considered shows by the two axes F1 and F2 (53.88% affinity), a grouping of the parameters into 1 nucleus (Figure 5). In this nucleus, there is a strong affinity between the increase in the contents of orthophosphates, MES, the color and the densities of *A. jandaei, A. schubertii, A. salmonicida, A. hydrophila* and the BHAMs at the sampling points Afamba 1, Lolo, Mefomo, Otoundoumba and Afamba 2 which are the surface water points (figure 5). This affinity is sometimes rare at groundwater level (Centre, CMA, Akam, Mekon and Chefferie).

4. Discussion

The study shows that the temperatures of the waters sampled from the town of Nkolafamba vary between 22°C and 31.2°C with an average value of 24.923 ± 2.05°C. This average is close to that obtained by [6]. In the waters of the same locality when these authors checked the microbiological and physicochemical quality of these waters. This temperature seems compatible with the activity of microorganisms [11]. In addition, very significant and positive correlations were observed between water temperature and ambient temperature ($P<0.01$; $r=0.524$). Indeed, the water temperature is directly dependent on the ambient temperature [11].

The pH values of the water during sampling ranged from 5.58 UC to 7.08 UC. This allows us to see that we thus pass from acidity to a very slight basicity. Similar values were obtained [15]. Following work carried out in the groundwater of the city of Yaoundé. Indeed, the acidity obtained would be due to the acidic nature of the soil in the region. As such, [23]. Point out that the pH of groundwater, although sensitive to various fluctuations, is not very different from that of the soil that contains it.

The electrical conductivity values fluctuated between 12 and 466 µs/cm. The low values sometimes observed of the electrical conductivity of the waters at certain stations could be explained by the low degradation of the organic matter present in the environment and would reflect the little polluted nature of these waters [24]. On the other hand, the high values of electrical conductivities recorded in other stations could be due to the infiltration of waste water in the boreholes studied or to the degradation of matter by microorganisms in the surface water. Indeed, the mineralization of groundwater depends on several parameters including the mineralogical nature of the rocks crossed, the contact time with the minerals, the speed of water circulation, the renewal time of the water in the aquifer [25].

The average value of dissolved CO_2 7.286 ± 6.06mg /L. According to [17], these levels are influenced by the climate and the seasons, as well as by the nature of the soil and the vegetation. In addition, the NO_3^- content is higher in June at the Center station (169 mg/L). This result differs from that obtained on the Konglo by [26] and on the Mbeme in Mbalmayo by [27]. According to the WHO grid (2004) these waters would therefore be unpolluted and favorable to the development of many biological groups and they can also be used for the production of water intended for WHO consumption [2].

On the bacteriological level, it appears that the waters of these wells harbor a bacterial community qualified as an opportunistic pathogen. During this study, in addition to the BHAMs which were isolated, several species of the Aeromonas genus, namely *A. hydrophila, A. jandaei, A. schubertii, A. media, A. salmonicida.* These results are similar to those obtained by [28] who, studying the dynamics of Aeromonas abundances in the groundwater of the city of Mfou, had isolated, in addition to the same species obtained in this study, the species *A. veronii biovar veronii*. The presence of bacteria of the *Aeromonas genera* at concentrations exceeding 200 CFU/100ml shows that the water analyzed is unfit for consumption [29]. Bacteria of the *Aeromonas* genera were more abundant in surface water than in groundwater. This high density of bacteria in surface waters (Afamba 1 and 2, Ottoundoumba, Lolo and Mefomo) could be explained by the availability and high levels of organic matter serving as food for microorganisms [17].The low density of bacteria in the boreholes compared to surface waters could be explained by the filtration exerted by the earth at the time of filtration and/or water percolation at the time of groundwater recharge [30]. In addition, BHAMs constitute the groups of bacteria for which the highest counts have been recorded. Indeed, according to [31], the enumeration of the aerobic bacterial flora aims to estimate the density of the general bacterial population.

Were sometimes noted between the contents of physicochemical parameters of the water sampled and the densities of bacteria (Table X). Thus it was observed a significant and positive correlation between the contents of dissolved CO_2 and the densities of *A. media* while the increase in conductivity, ambient temperatures and that of the water, and the contents of NO_3^- decrease bacterial densities. In this regard, [31].) are of the opinion that in a given environment, increases in pH sometimes favor the development of *Pseudomonas* and *Aeromonas* as well as the abundance of faecal coliforms and faecal enterococci.

The results of the antibiogram tests mostly show Aeromonas *resistant* to the antibiotics tested, although there may be intermediate reactions or the bacteria are sometimes sensitive depending on the species identified and the water point sampled (tables VI and VII). Similar results were obtained by [13] on *Pseudomonas* isolated from Douala and Yaoundé rivers ; and by [32], who studied the importance of the properties of the aquatic biotope in Yaoundé on the susceptibility of certain Enterobacteriaceae to antibiotics from the aminoglycoside family. This strong resistance is due to the constant presence of residues of active substances (fertilizers, detergents, drugs) in the water, thus increasing the contact time and bacterial resistance according to the one health approach [29].

Correlations were noted between the diameters of inhibition of the bacteria after the antibiogram and the physicochemical variables of the water sampled (Table XI). Antibiotic resistance is a function of the place of isolation of the bacterial species considered and of the abiotic variables of the place of isolation [32].

5. Conclusion

The present work aims to evaluate the antibiotic sensitivity of bacteria of the genus *Aeromonas* isolated from underground water points and some surface water points in the Commune of Nkolafamba (Centre Cameroon). Overall, it emerges that the waters analyzed are most of the time used for drinking (manually powered borehole water or groundwater) and for washing up, laundry and plant irrigation for surface water. These waters harbor Heterotrophic Aerobic Mesophilic Bacteria and five species of bacteria of the genus *Aeromonas* pathogenic for humans. Due to the constant exposure of these bacteria to detergents, fertilizers or antibiotic residues, they usually show resistance to the antibiotics constantly used in the city, thus limiting the development of the latter. This result makes it possible to recommend that the health authorities carry out antibiogram analyzes before any health prescriptions. 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, Delphine Mvondo Nga, Samuel Davy Baleng conceptualized, analyzed the data and prepared the manuscript. Lucie Leme Banock, Serge Ronny Ott Song and Awawou Manoure Njoya in collecting data, in analyzing and interpreting. The was supervised by Moïse Nola. All authors have read, agreed and approved the final manuscript.

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