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Assessment of the potential effect of some streams properties on the isolated *Aeromonas hydrophila* strains susceptibility against some β-Lactams and Sulfamids

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The isolation and characterization of *A. hydrophila* strains were carried out a given stream located in the equatorial zone in Cameroon (Central Africa), during the mild rainy season and the mild dry season. The water physicochemical parameters measured were temperature, pH, electrical conductivity, color, turbidity and suspended solids. It has been noted that bacterial abundances as well as the values of physicochemical parameters undergoes temporal variations. Antimicrobial susceptibility tests showed that the means diameters of inhibition with Oxacillin, Ceftriaxone, Penicillin, Sulfamethoxazole-trimethoprim, Chloramphenicol, Imipenem, Amoxicillin-Clavulanic acid and Cefazolin were 6.44, 21, 6.63, 9.58, 15.37, 22.18, 14.29 and 10.26 mm respectively. 100% of strains tested were resistant to Penicillin, 89.47 % resistant to Oxacillin, 68.42% resistant to Sulphamethoxazole-trimetroprim and 63.15% were resistant to Cefazolin. There were 54.60% cases of antibiotic resistance, 24.34 % of intermediate sensitivity and 21.05% of sensitive strains against the tested antibiotics. The encoded redundancy analysis indicates that the percentage of the variation explained on the canonical axes is of 47.8% for the F1 axis and 25.9% for the F2 axis, for a cumulated percentage of 73.7%. The suspended solids, the Ceftriaxone and Chloramphenicol are correlated to the F1 axis in positive coordinates, and the Temperature and Amoxicillin-Clavulanic acid in negative coordinates. The correlation test between the water physicochemical characteristics and the inhibition diameters of antibiotics showed some significant correlations (P<0.05). The regulation of bacterial genes would be regulated by complex mechanisms. Although many factors implied are linked to the bacterial cell, others may belong to the closed environment.

Key words: *A. hydrophila* aquatic strains, antibiotic, antimicrobial susceptibility, water chemical characteristics.

Aeromonas hydrophila is an emerging aquatic pathogen bacterium, widely distributed in the environment. It is also part of the normal bacterial flora of many animals (Stojanov *et al.*, 2010). According to Graf (2015), the genus *Aeromonas* is now

classified within the family *Aeromonadaceae* and consists of 14 different species, one of which is *A. hydrophila*. It is a Gram-negative, oxidase-positive bacillus that is a common freshwater and food-borne pathogen that can cause enterocolitis, bacteremia, meningitis, and soft tissue infections. *A. hydrophila* is considered as a cause of several disease conditions in cold-blooded animals as well as in warm-blooded animals (Gosling, 1996; Graf, 2015). It secretes many extracellular proteins associated with pathogenicity and environmental adaptability (Ashraf, 2010). Since the wide distribution of *A. hydrophila* is probably a consequence of its high capacity to adapt to different environments, it would seem that its genetic and phenotypic diversity are from natural result.

Previous studies of antibiotic susceptibility of *A. hydrophila* indicated the existence of many strains highly resistant to some antibiotics applied in clinical practice. Some reports showed that all of *A. hydrophila* strains isolated from water, food, clinical specimens and other sources are not susceptible to many antibiotics. Krovacek *et al*. (1989) and Rossi *et al*. (2006) noted that some *A. hydrophila* strains are susceptible to chloramphenicol, neomycin, sulfameth-oxazol, streptomycin and sulfamethoxazol-trimethoprim. Furthermore, studies on the antimicrobial susceptibility of mesophilic *Aeromonas* isolated from two European rivers showed that almost all the isolates were resistant to the antibiotics tested including nalidixic acid, tetracycline, tobramycin, cotrimoxazol, fosfomicin, cefotaxime, chloramphenicol, gentamicin and most of the nalidixic acid resistant strains were susceptible to fluoroquinolons (Goni-Urriza *et al*., 2000). Castro-Escarpulli *et al*. (2003) and Harnisz *et al*. (2011) noted that the best antimicrobial effect against *A. hydrophila* strains are of the first generation quinolones and second and third generation cephalosporins.

The immediate aquatic environment of the bacterial cell exerts on it variable effects. Various biotic and abiotic factors are likely to influence its development as well as its productivity (Wiebe *et al*., 1992; Sander and Kalff, 1993). The chemical characteristics of aquatic environment influence the bacterial distribution, the amplitude of this influence varying with the considered bacterial species (Fowle and Fein, 2000; Yee *et al*., 2000). The bacterial feeling at the chemical environment depends upon the number and the properties of groups of functional sites on its surface, and the disposal sites number can vary with chemical features of the biotope (Fein *et al*., 1997). Bacterial feeling sometimes depends upon the presence or the absence of some external polymer synthesized by the bacterium itself and which serve as chemical communication pathway (Jucker *et al*., 1998; Kolter and Losick, 1998). Few studies have so far been carried out on the impact of these water physicochemical parameters on the bacteria susceptibility against antibiotics. The present study aimed at assessing the effect of some abiotic parameters on the susceptibility of *A. hydrophila* isolated from a river in Yaounde (Cameroon) to some Beta-Lactams and Sulfamids.

MATERIAL AND METHODS

Description of cells trains collection site

Cells strains were collected from the Olezoa stream at the Mfoundi River basin which is the main water network irrigating the Yaounde region (Cameroon, Central Africa). Yaoundé region is located at 3°52'N latitude and 11°32'E longitude, with an average altitude of 760m. The climate is tropical sub-equatorial. It has four alternating rainy and dry seasons (Succhel, 1988); a mild rainy season from April to June, a mild dry season from July to August, a peak rainy season from September to November and a peak dry season from December to March of the following year. The duration of a season can vary from one year to another. The annual mean value of rainfall is 1576 mm. The annual temperature varies from 20 to 31°C. The soil is ferrolateritic and acidic,

with a pH values generally lower than 6 (Bachelier, 1959).

Water Sampling and analysis

Water samples were collected at one point on the Olezoa stream. Samples were manually collected at 15 cm below the surface in 100 ml sterile glass bottles and in polyethylene clean bottles of 500 ml, once every two weeks from February to July 2014. This period was chosen because it covered the mild rainy season and the mild dry season.

The samples in the glass bottles were used for the bacteriological analyses while those in polyethylene bottles were used for physicochemical analyses. The samples were then transported to the laboratory in dark refrigerated conditions for laboratory analyses. The time lapse between the sample collection and laboratory analyses was in all cases lower than 2 hours. The physicochemical parameters measured were water temperature, pH, electrical conductivity, color, turbidity and suspended solids (SS). The analyses were carried out using standard methods (Rodier, 1996; APHA, 1998).

The bacteriological parameter considered was *Aeromonas hydrophila*. The spreading surface technique was used for bacterial isolation using the Ampicillin-Dextrin Agar culture medium poured in Petri dishes (Marchal *et al*., 1991). A volume of 100 µl of water sample was spread on culture medium and after 24h of incubation at 37°C, large yellow convex colony forming units (CFUs) were counted. Results were expressed as number of CFU/100 µl. Each yellow CFU was subsequently identified after a sub-culture on a standard agar medium, according to Holt *et al.* (2000).

Antibiotics susceptibility tests

Antibiotics susceptibility tests were done by the method of disc diffusion on Müller Hinton medium according to the recommendations of the "Comité de l'Antibiogramme de la Société Française de Microbiologie" (CA-SFM) (Soussy *et al*., 2012). The antibiotics disc used were chosen depending to the naturally susceptibility against *A. hydrophila*. The discs used against which *A. hydrophila* is naturally sensitive were Trimethoprim-Sulfamethoxazole (25µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Imipenem (10µg); and those used against which *A. hydrophila* is naturally resistant were Penicillin G (10UI), Oxacillin (1µg), Cefazolin (30µg), Amoxicillin-Clavulanic acid (30µg). The results were recorded as resistant, sensitive or intermediate. The diameters of inhibition were then measured using the calipers, according to the interpretative of the CA-SFM (Abulhamd, 2010; Soussy *et al*., 2012).

Data analysis

The Pearson correlation test was achieved from the data between abiotic and bacteriological parameters in order to assess the possible relations that exist between the considered variables. The redundant analyses were also done in order to appreciate the possible ecological role of the variation of the abiotic factors. Statistical analyses were performed using SPSS version 16.0 and CANOCO Version 4.5.

RESULTS

Physicochemical parameters

It was noted that values physicochemical parameters during the study period underwent temporal variations, from one sampling campaign to the other (Figure 1). The water temperature varied from 23 to 26°C, the lowest value being recorded during the sampling campaigns C3, C4, C7 and C9, whereas the highest was recorded during the sampling campaign C1 (Figure 1). In the same way, the pH value varied from a light acidity to a light alkalinity, between 6.60 and 7.43 UC. The highest value was recorded in the sampling campaign C9 and lowest during the C6. The electrical conductivity fluctuated between 310 and 663µS/cm. It was noted that the water color values (166.67 Pt.Co) remained nearly constant during the sampling campaigns C4, C5 and C6. The highest value was recorded at sampling campaign C10 and the lowest at

C3 (Figure 1). The suspended solids content in water during the study period fluctuated between 9 and 97 mg/l. The water turbidity values fluctuated between 8 and 63 FTU. On the whole, the turbidity varied from one sampling campaign to the other; the highest value was recorded during the sampling campaign C10 and the lowest during the C1 (Figure 1).

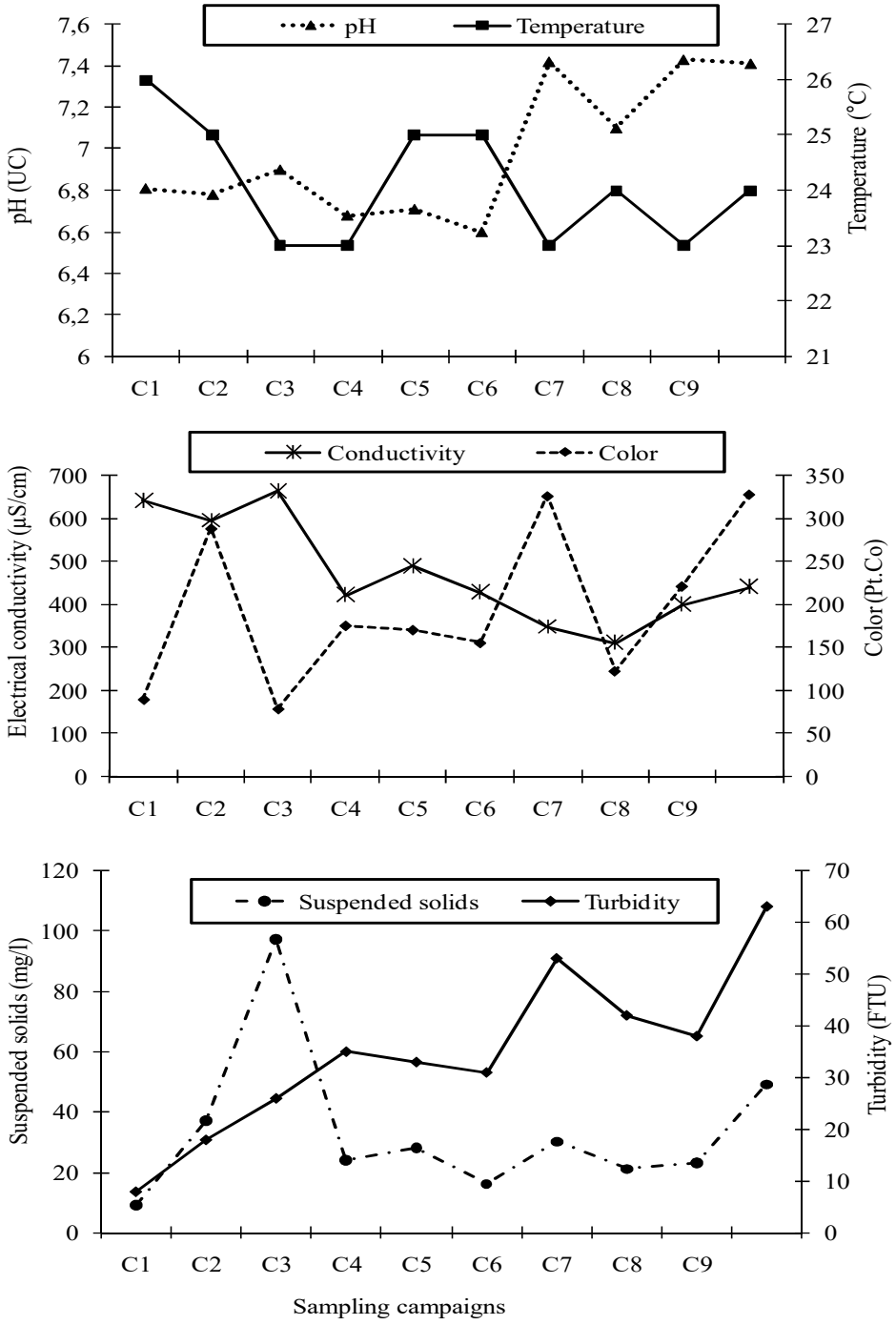

Figure 1. Variation of pH, temperature, electric conductivity, color, suspended solids and turbidity values with respect to the sampling campaigns.

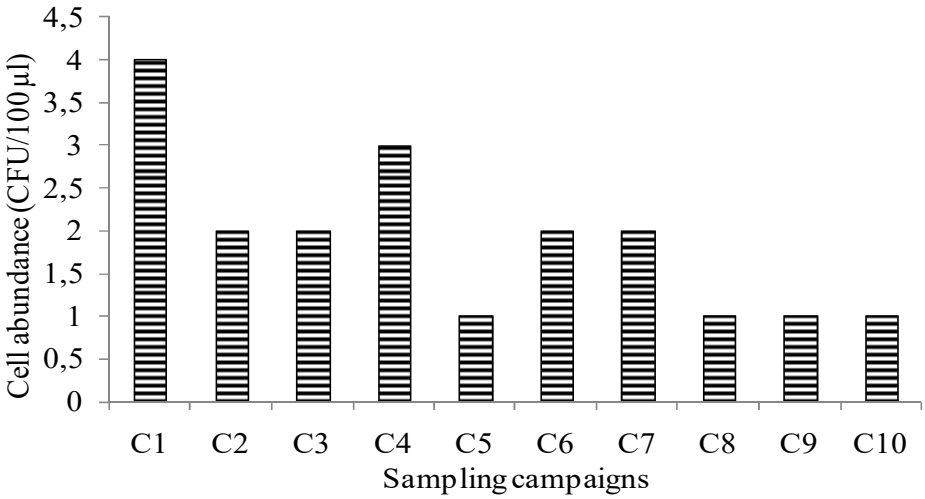

Figure 2. Variation of the abundance of *A. hydrophila* with respect to the sampling campaigns

Table 1. Correlation coefficients between the abiotic parameters and *A. hydrophila* abundances

Bacteriological parameter	Abiotic parameters					
	pH	E. cond	Temp	S.S.	Color	Turbidity
Cell abundances	- 0.463	0.493	0.315	- 0.204	- 0.354	**- 0.633***

*: P<0.05; df = 5; Temp: temperature; S.S.: suspended solids; E cond: Electrical conductivity

Table 2. Correlation coefficients between the diameters of inhibition of each antibiotic and water abiotic parameters

Water abiotic parameters	Antibiotics							
	OX	CRO	P	SXT	C	IMP	AMC	CZ
Conductivity	-0.207	0.000	-0.074	0.122	0.155	-0.059	0.312	0.378
pH	**0.585****	0.392	-0.120	-0.168	0.107	**-0.467***	-0.207	0.081
Temperature	-0.215	-0.289	0.108	0.067	-0.188	0.015	**0.513***	0.361
SS	0.087	0.287	-0.171	0.102	0.331	-0.113	-0.281	0.008
Color	0.307	0.171	0.008	-0.363	-0.065	-0.245	-0.142	0.014
Turbidity	0.404	0.206	-0.020	-0.165	-0.109	-0.114	-0.365	-0.278

: P <0.01; *: P <0.05. df = 12 ; SS: Suspended Solids; OX: Oxacillin. CRO: Ceftriax**one. P: Penicillin. SXT: Sulfamethoxazol**e**-trimethoprim. C: Chloramphenicol. IMP: Imipenem. AMC: Amoxicillin- Clavulanic acid. CZ: Cefazolin.

Bacteriological parameters

The abundances of *A. hydrophila* also varied from one sampling campaign to the other. They ranged from 1 CFU/100 µl recorded at the sampling campaigns C5, C8-C10 to 4 CFU/100 µl registered during the sampling campaign C1 (Figure 2).

Antimicrobial susceptibility tests showed a high incidence of resistance of the isolates against several antibiotics (Figure 3). There were 54.60% cases of antibiotic resistance, 24.34 % of intermediate sensitivity and 21.05% cases of sensitive bacterial strains against the tested antibiotics. It was noted that 100% of the bacteria tested were resistant to Penicillin, 89.47 % resistant to Oxacillin, 68.42% resistant to Sulphamethoxazole-trimetroprim and 63.15% were resistant to Cefazolin.

Ceftriaxone showed the highest efficacy against the bacterial strains tested (63.15% sensitive and 21.05% resistant). Other effective antibiotic was Imipenem (57.89% sensitive and 5.26% resistant) (Figure 3). The diameters of inhibition of a given antibiotic varied in most cases from one sampling campaign to another. The diameters of inhibition with Oxacillin, Ceftriaxone, Penicillin, Sulfamethoxazole-trimethoprim, Chloramphenicol, Imipenem, Amoxicillin-Clavulanic acid and Cefazolin varied respectively from 5.5 to 12.5, from 11 to 29, from 5 to 8, from 5 to 20, from 5 to 23, from 15 to 30, from 6 to 21 and from 6 to 15 mm. The calculated values of the mean diameters of inhibition were 6.44, 21, 6.63, 9.58, 15.37, 22.18, 14.29 and 10.26 mm respectively (Figure 3).

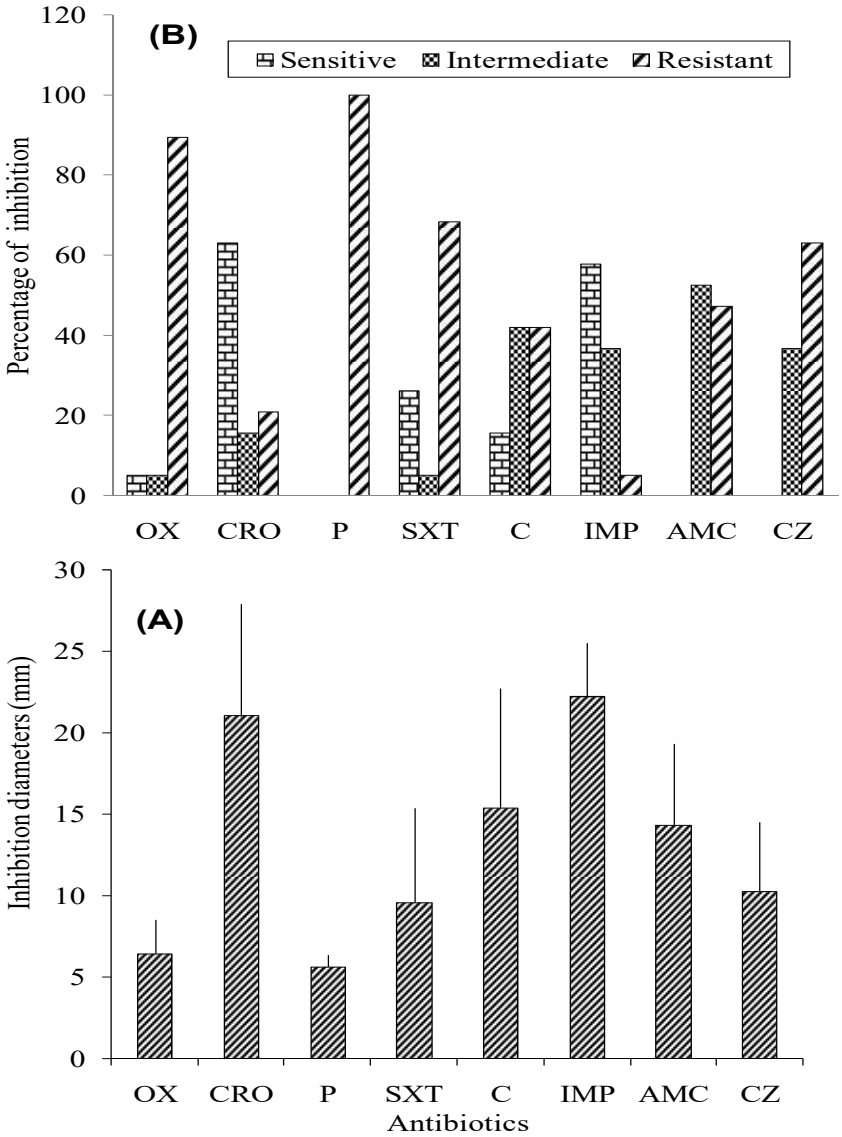

Figure 3. Distribution of bacterial susceptibility percentage to antibiotics (**A**), and the mean value (with standard error) of inhibition diameter of each antibiotic used (**B**) (OX: Oxacillin; CRO: Ceftriaxone; P: Penicillin; SXT: Sulfamethoxazole-trimethoprim; C: Chloramphenicol; IMP: Imipenem; AMC: Amoxicillin-Clavulanic acid; CZ: Cefazolin).

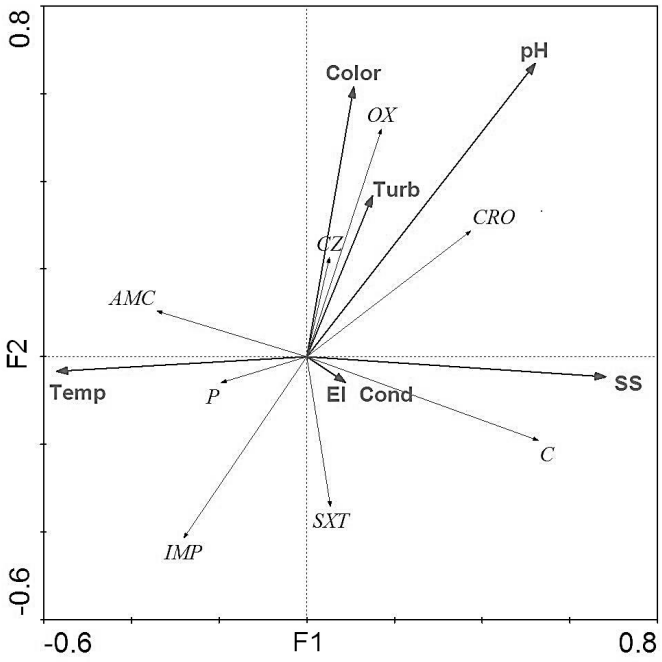

Figure 4. Biplot representation of the redundancy analysis between abiotic parameters and the diameters of antibiotics inhibition (OX: Oxacillin; CRO: Ceftriaxone; P: Penicillin; SXT: Sulfamethoxazole-trimethoprim; C: Chloramphenicol; IMP: Imipenem; AMC: Amoxicillin-Clavulanic acid; CZ: Cefazolin)

Correlations amongst the considered parameters

The Pearson correlation test showed during the study period the rise in turbidity led to a significant decrease of the abundance of *A. hydrophila* ($P<0.05$) (Table 1).

The Pearson correlation test was also carried out between the diameters of antibiotics inhibition and water abiotic factors. The results showed that there is a positive and significant relationship between the *A. hydrophila* susceptibility against Oxacillin and pH ($p<0.01$) and between susceptibility against Amoxicillin-clavulanic acid and temperature (Table 2). Therefore, a rise in turbidity and temperature seems to affect the susceptibility of *A. hydrophila* to these antibiotics. On the contrary, a negative and significant relationship ($P<0.05$) between the *A. hydrophila* susceptibility against Imipenem and pH was noted. A rise in water pH seems to render the bacteria less susceptible to this antibiotic (Table 2).

The encoded results of the redundancy analysis indicate that the percentage of the variation explained on the canonical axes is of 47.8% for the F1 axis and 25.9% for the F2 axis, for a cumulated percentage of 73.7%. The suspended solids, the Ceftriaxone and Chloramphenicol are correlated to the F1 axis in positive coordinates, and the Temperature and Amoxicillin-Clavulanic acid in negative coordinates. As for the F2 axis, the Color, Turbidity, pH and the Oxacillin are correlated in positive coordinates, and the Imipenem and the Sulfamethoxazole-trimethoprim in negative (Figure 4).

With regard to the relationships amongst the considered factors, it is noted that some positive relationships exist between the water temperature and the sensitivity of *A. hydrophila* to Amoxicillin-Clavulanic acid, as well as between the

water turbidity and the sensitivity to the Oxacillin. Moreover, a positive relationship exists between the pH and the sensitivity of the bacteria to the Ceftriaxone and negative relationship between the bacterial sensitivity to the Imipenem and pH.

DISCUSSION

It has been noted that the bacterial abundance and values of physico-chemical parameters undergoes temporal fluctuation (Figures 1-2). The turbidity of water is caused by colloidal or suspended matters as clay, silt, fine particles of organic or inorganic matters, plankton and other microscopic organisms (Rodier, 1996). Water color entirely results from the extraction of the organic matters in decomposition, as well as the dissolution of some ions as iron, the manganese and the copper (Olanezuk-Neyman and Bray, 2000), although some dissolved ions can favor bacterial growth whereas others can rather facilitate the increase of the cell adherence speed (Pelmont, 1993),

The relationship between bacterial abundances and abiotic parameters showed that the increase of the turbidity is correlated significantly to the reduction of cell abundances of *A. hydrophila*. This could be explained by the entry of the bacterium in viable but non cultivable state. However, numerous studies that put in evidence a link between the turbidity and the presence of microorganisms (bacteria, mushrooms, yeasts and protozoa) and virus in drinking water, the microbial abundance being relatively lower in general in water having a low turbidity (Bertrand *et al*., 2015). The abundance of *Aeromonas* sp and fecal coliforms is sometimes significantly correlated to the decease of the water turbidity when the water temperature is relatively higher (Maalej *et al*., 2002).

It has been noted that the isolated strains are resistant to a first generation cephalosporin (Soussy *et al*., 2012). The isolated strains showed a high rate of resistance to Chloramphenicol and Sulfamethoxazole-trimethoprim, antibiotics to which the bacterium is generally sensitive. This loss of sensitivity to these antibiotics could be due to a decrease of the membrane permeability or to the acquirement of resistant plasmids or transposons (Goni-Urriza *et al*., 2000). It has been indicated that the transfer of multiresistant plasmids is a phenomenon that belong to the environment and can occur between bacterial strains of human, animal and fish origin, that are unrelated either evolutionarily or ecologically even in the absence of antibiotics (Kruse and Sorum, 1994). The multiresistant character is noted within *A. hydrophila* strains isolated. This resistance may be associated to the production of three chromosomal enzymes oxacillinase, cephalosporinase and carbapenemase of inducible type with coordinated expression. However, these results differ from those of many authors who recorded a low rate of resistance of the species *A. hydrophila* to the Phenicols, Rifamycines and Sulfamids (Hamze *et al*., 1998; Ashraf, 2010). In addition, El Mejri *et al*. (2008) showed that *A. hydrophila* lost its sensitivity to Chloramphenicol, Sulfamethoxazole-trimethoprim and Oxalinic acid after a stay in waste water before stay in marine water microcosm.

The correlation between the diameters of inhibition and water physicochemical factors showed that the variations of the pH are significantly ($P<0.01$) and positively correlated to the variations of the diameter of inhibition by Oxacillin. Furthermore, the redundancy analysis revealed a positive relationship between the pH and Ceftriaxone, meaning that the increase of pH affect *A. hydrophila* by increasing its sensitivity to these antibiotics. However, a significant and negative relationship ($P<0.05$) has been observed between the variations of the pH and the diameters of inhibitions against Imipenem. It means that the neutral pH could reduce the sensitivity of *A. hydrophila* to the Imipenem. The antibiotic susceptibility of aquatic microorganisms against antibiotics may be impacted by the water pH value. Indeed Mercier *et al.* (2002)

showed that the bactericidal activity of the Oritavancin against the vancomycin-resistant *Enterococcus faecium* strains was significantly high when these were tested at pH 7.4 and 8.0 ($P<0.05$).

It has been indicated that the inhibition of microbial development by pH is not due to the direct result of H^+ or OH^- concentrations, but to the indirect influence of pH on the penetration into the microbial cell, by some unusual compounds in the medium. This indirect action of pH modifies the assimilation of different mineral or organic nutrients by the bacteria (Nola *et al*., 2002). These compounds can subsequently cause genetic mutations in the bacterial cell, causing variability of genes and phenotypes in the presence of antibiotics. Olson (1993) indicated that bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence. While the physiological and biological consequence of the pH-induced response is clear, the mechanism by which the transcription/translation machinery is signaled is not yet clear and seems to remain a mystery.

Working on *Lactobacillus acidophilus*, Kullen and Klaenhammer (1999) indicated that the increase in atp mRNA induced by low pH was accompanied by an increase in the activity of the enzyme in membrane extracts. Primer extension analysis of RNA from cultures revealed that the transcriptional start site did not change position as a function of culture pH or time after exposure to pH 3.5. In addition, Martin-Galiano *et al.* (2001) indicated that many enzymes as the promoter of the operon encoding of *Streptococcus pneumonia* are inductible by pH value of the environment.

The significant correlation between the water temperature and the diameter of inhibition to Amoxicillin-Clavulanic acid would mean that a decrease of the water temperature could reduce the sensitivity of *A. hydrophila* strains against Amoxicillin-Clavulanic acid. Virulence gene expression in most bacteria is a highly regulated phenomenon, affected by a variety of parameters including osmolarity, pH, ion concentration, iron levels, growth phase, and population density (Konkel and Tilly, 2000); it is also regulated by temperature, which acts as an on-off' switch in a manner distinct from the more general heat-shock response.

However, regulation by thermal stimulus occurs through a wide variety of mechanisms, which generally act in conjunction with (or are modulated by) additional controls for other environmental cues oxygen tension and DNA damage (Marceau, 2005). According to Hurme and Rhen (1998), temperature-mediated regulation occurs at the level of transcription and translation. Supercoiling, changes in mRNA conformation and protein conformation are all implicated in thermosensing. mRNA melting also act as a thermosensing mechanism in various contexts. In addition, Sabath *et al*. (2013) working on growth temperature and genome size in bacteria noted that with increasing habitat temperature and decreasing genome size, the proportion of genomic DNA in intergenic regions decreases. Genome size may be an indirect target of selection due to its association with cell volume. Other more, they noted that known changes in cell structure and physiology at high temperature can provide a selective advantage to reduce cell volume at high temperatures.

Some recent studies showed that the antibiotic profile of *A. hydrophila* is modified when it is submitted to different environmental conditions of stress. Indeed, El Mejri *et al.* (2008) showed that *A. hydrophila* B3 strain initially resistant to 6 different antibiotics (Amoxicillin, Oxacillin, Furans, Rifampicin, Streptomycin and Novobiocin), after stay in different conditions of stress (sea water, mineral water and sea water + solar radiation), loses

its resistant character against some antibiotics. When working on the regulatory elements implicated in the environmental control of *invasin* expression in enteropathogenic *Yersinia,* Heroven *et al.* (2007) used mutagenesis and gene bank screens to identify regular components modulating the levels of *invasin* and *RovA* and found that the invasin and rovA genes were both subjected to silencing by the nucleoid-associated protein *H-NS*. Under inducing conditions, *RovA* appears to disrupt the silencer complex, through displacement of *H-NS* from an extended AT-rich region located upstream of the *rovA* promoters.

Ramalivhana *et al.* (2009) studied antibiotic résistance of 300 *A. hydrophila* strains collected from water and stool samples in South Africa. They found that most strains were susceptible to Aminoglycosides, Carbapenems, Mono-bactams, Cephalosporins and Beta-lactam/Beta-lactam inhibitors and almost all were resistant to ampicillin. However, fluvial waters receive human and animal wastewater discharges, which are expected to contain antimicrobial agents likely to exert a selective pressure, and also commensal resistant bacteria, capable of transferring their resistances to autochthonous bacteria.

CONCLUSION

The physicochemical characteristics of the stream water sampled in Cameroon undergo temporal fluctuation. This streams harbors *A. hydrophila* strains which abundances also undergoes temporal variation. The isolated bacterial strains seem resistant against some β-Lactams and Sulfamids and sensitive against others. Relationships between the water physicochemical characteristics registered and the inhibition diameters of antibiotics used showed some significant correlation. This would means that regulation of bacterial genes is regulated by complex mechanisms. Although many factors implied are linked to the bacterial cell, others may belong to the closed environment.

REFERENCES

Abulhamd A. 2010. Genetic diversity and antimicrobial susceptibility of motile aquatic aeromonads. International Journal of Chemical Engineering and Applications. 1(1): 90-95.

APHA. 1998. *Standard methods for the examination of water and wastewater*. 20th Ed., American Public Health Association /American Water Works Association /Water Environment Federation, Washington DC, USA.

Ashraf A. 2010. Genetic diversity and antimicrobial susceptibility of motile aquatic aeromonads. International Journal of Chemical Engineering and Applications. 1(1): 90-95.

Bachelier G. 1959. Etude pédologique des sols de Yaoundé : contribution à l'étude de la pédologie des sols ferralitiques. Agronomie Tropicale. 14 : 279–305.

Bertrand JC, Caumette P, Lebaron P, Matheron R, Normand P and Sime-Ngando T. 2015. *Environmental microbiology: Fundamentals and applications*, London: Springer.

Castro-Escarpulli G, Figueras MJ, Aguilera-Arreola,G, Soler L, Fernandez- Rendon EG, Aparicio O, Guarro J, and Chacon MR. 2003. Characterisation of *Aeromonas* spp isolated from frozen fish intended for human consumption in Mexico. International Journal of Food Microbiology. 84: 41–49.

El Mejri S, El Bour M, Boukef I, Mraouna R and Boudabbous A. 2008. Effect of previous incubation of *Aeromonas hydrophila* in wastewater prior to its transfer into marine water microcosms. Journal of Water Science. 21(3): 259-266.

Fein JB, Daughney CJ, Yee N and Davis TA. 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. Geochimica et Cosmochimica Acta. 61:3319-28.

Fowle DA and Fein JB., 2000. Experimental measurements of the reversibility of

metal–bacteria adsorption reactions. Chemical Geology. 168:27-36.

Goni-Urriza M, Pineau L, Capdepuy M, Roques C, Caumette P and Quentin C. 2000. Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. Journal of Antimicrobial Chemotherapy. 46 (2): 297-301.

Gosling PJ. 1996. *Aeromonas* species in disease of animals. In *The Genus Aeromonas,* Eds. B. Austin, M. Altwegg, P.J. Gosling and S. Joseph, Wiley and Sons, Ltd, pp. 175–196.

Graf J. 2015. *Aeromonas*, UK: Caister Academic Press.

Hamze M, Dabboussi F and Izard D. 1998. Sensibilité à 23 antibiotiques de 83 souches d'*Aeromonas hydrophila* isolées d'eaux libanaises. Cahiers Association Scientifique Européenne Eau et Santé. 3: 91-96.

Harnisz M, Gołas I and Pietruk M. 2011.Tetracycline-resistant bacteria as indicators of antimicrobial resistance in protected waters-The example of the Drweca River Nature Reserve (Poland). Ecological Indicator. 11: 663-668

Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST. 2000. *Bergey's Manual of Determinative Bacteriology*, Philadelphia: PA Lippincott Williams and Wilkins,.

Heroven AK, Böhme K, Tran-Winkler H and Dersch P. 2007. Regulatory elements implicated in the environmental control of invasin expression in entero-pathogenic *Yersinia*. Advances in Experimental Medicine and Biology. 603:156-66.

Hurme R and Rhen M. 1998. Temperature sensing in bacterial gene regulation-what it all boils down to. Molecular Microbiology. 30(1):1-6.

Jucker BA, Zehnder AJB and Harms H. 1998. Quantification of polymer interactions in bacterial adhesion. Environmental Science and Technology. 32: 2909-2915.

Kolter R and Losick R. 1998. One for all and all for one. Science. 280: 226-227.

Konkel ME and Tilly K. 2000. Temperature-regulated expression of bacterial virulence genes. Microbes Infections. 2(2): 157-66.

Krovacek K, Peterz M, Faris A and Mansson I. 1989. Enterotoxigenicity and drug sensitivity of *Aeromonas hydrophila* isolated from well water in Sweden: A case study. International Journal of Food Microbiology. 8: 149-154

Kruse H and Sorum H. 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Applied and Environmental Microbiology. 60(11): 4015-4021.

Kullen MJ and Klaenhammer TR. 1999. Identification of the pH-inducible, proton-translocating F1F0-ATPase (atpBEFHAGDC) operon of *Lactobacillus acidophilus* by differential display: gene structure, cloning and characterization. Molecular Microbiology. 33(6):1152-1161.

Maalej S, Mahjoubi A, Kammoun A and Barhrouf-Fadhila A. 2002. Motile *Aeromonas*: which spatial and temporal evolution in an urban effluent and coastal marine environment? Revue des Sciences de l'Eau. 15(1): 273-287.

Marceau M. 2005. Transcriptional regulation in *Yersinia*: an update. Current Issues in Molecular Microbiology. 7(2):151-77.

Marchal N, Bourdon JL and Richard C. 1991. *Les milieux de culture pour l'isolement et l'identification biochimique des bactéries*, Paris: Doin.

Martin-Galiano AJ, Ferrandiz MJ and de la Campa AG. 2001. The promoter of the operon encoding the F0F1 ATPase of *Streptococcus pneumoniae* is inducible by pH. Molecular Microbiology. 41(6):1327-1338.

Mercier R-C, Stumpo C and Rybak MJ. 2002. Effect of growth phase and pH on the *in vitro* activity of a new glycopeptide, oritavancin (LY333328), against *Staphylococcus aureus* and

Enterococcus faecium. Journal of Antimicrobial Chemotherapy. 50: 19-24.

Nola M, Njine T, Djuikom E and Sikati Foko V. 2002. Faecal coliforms and faecal streptococci community in the under-ground water in an equatorial area in Cameroon (Central Africa): The importance of some environmental chemical factors. Water Research. 36: 3289-3297.

Olanezuk-Neyman K and Bray R. 2000. The role of physico-chemical and biological processes in manganese and ammonia nitrogen removal from groundwater. Polish Journal of Environmental Studies. 9: 91-96.

Olson ER. 1993. Influence of pH on bacterial gene expression. Molecular Microbiology. 8(1):5-14.

Pelmont J. 1993. *Bactérie et environnement-Adaptations Physiologiques*, Grenoble : Presses Universitaires de Grenoble.

Ramalivhana JN, Obi CL and Moyo SR. 2009. Antimicrobial susceptibility testing of *Aeromonas hydrophila* isolated from Limpopo Province, South Africa using VITEK 2 system, Micro Scan Walk Away, disk diffusion and E-test method. African Journal of Microbiology Research. 3(12): 903-913.

Rodier J. 1996. *L'analyse de l'eau*, Paris: Dunod.

Rossi JOD, Amaral LA, Nader Filho A, Schocken-Iturrino RP. 2006. Bacteria of the genus *Aeromonas* in different locations throughout the process line of beef slaughtering. Revista Portuguesa de Ciencias veterinarias. 101: 125-129.

Sabath N, Ferrada E, Barve A and Wagner A. 2013. Growth temperature and genome size in bacteria are negatively correlated, suggesting genomic streamlining during thermal adaptation. Genome Biology and Evolution. 5(5): 966-977.

Sander BC and Kalff J. 1993. Factors controlling bacterial production in marine and freshwater sédiments. Microbial Ecology. 26: 79-99.

Soussy CJ, Bonnet R, Caron F, Cavallo JD, Chardon H, Chidiac C, Courvalin P, Drugeon H, Dubreuil L, Jarlier V, Jehl F, Lambert T, Leclercq R, Nicolas-Chanoine MH, Plesiat P, PloyMC, Quentin C, Soussy CJ, Varon E and Weber P. 2012. *Comité de l'Antibiogramme de la Société Française de Microbiologie. Recommandations*. (Online). http://www.sfm-microbiologie.org /UserFiles/files/casfm/CASFM_2012.pdf (accessed 25th May 2015)

Stojanov I, Plavsa N, Stojanovic D, Ratajac R, Prodanov Radulovic J, Pusic I, Kapetanov M. 2010. Susceptibility of *Aeromonas Hydrophila* isolates to antimicrobial drugs. Lucrari Stiinłifice Medicina Veterinara. 43 (1): 132-136.

Succhel B. 1988. *Les climats au Cameroun*, Université de Bordeaux III, France, Thèse de Doctorat d'Etat.

Wiebe WJ, Sheldon WM, Pomeroy LR. 1992. Bacterial growth in the cold: evidence for an enhanced substrate requirement. Applied and Environmental Microbiology. 58: 359-364.

Yee N, Fein JB and Daughney CJ. 2000. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. Geochimica et Cosmochimica Acta. 64: 609-617.