University of Tehran Press

Pollution

Print ISSN: 2383-451X
Online ISSN: 2383-4501

https://jpoll.ut.ac.ir/

National Survey of Stream Water Quality Revealing Threats to Antibio-Resistant Bacteria, Antibiotic Residues and Heavy Metals in Benin

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Article Info

Article type:
Research Article

Article history:
Received: 13 Sep 2022
Revised: 28 Nov 2022
Accepted: 17 Jan 2023

Keywords:
Antibiotic resistant Bacteria
Antibiotic residues
Heavy metals
Streams
Benin

ABSTRACT

Benin's waterways are affected by several forms of pollution that are linked in particular to anthropic activities. This study aims to detect the presence of antibiotic residues, the frequency of antibiotic resistant bacteria and the levels of heavy metals in Benin's waterways. 160 surface water samples from streams in Benin were collected. They were filtered by the membrane filtration method, then incubated on different media. The isolated bacterial species were identified by API 20E gallery and specific biochemical tests. After detection of the resistance profile of the latter, the antibiotic residues were quantified in the samples by the ELISA technique on plate and the physicochemical analyses were performed by Multi 3630 IDS SET KS2 multimeter. Finally, heavy metal levels were detected by the MERCK test kit method specific to each metal. The bacterial species mostly identified were *Klebsiella pneumoniae* (56.59%), *Klebsiella* spp. (18.68%), *Enterobacter* spp. (12.63%). The most abundant resistance of bacterial strains was to amoxicillin + clavulanic acid (92%), followed by metronidazole (86%). Metronidazole was the antibiotic with the highest residue concentration in the samples (6.578 to 6.829 µg/L), followed by ciprofloxacin (2.142 to 9.299 µg/L). Benin streams contain heavy metals such as mercury (0.454±0.129 µg/L), lead (0.040±0.50 mg/L), zinc (6.120±16.017 mg/L), nickel (0.155±0.233 mg/L) and cadmium (0.154±0.132 mg/L). The analysis of the physico-chemical parameters showed that, apart from electrical conductivity, all parameters comply with Beninese and World Health Organization standards. Actions must be taken to clean up these rivers to preserve the integrity of aquatic ecosystems in Benin.

Cite this article: Gbotche, E., Camille Houssou Quenum, M., Victorien Dougnon, T., Ogunlaja A., Klotoe, J.R., Fabiyi, K., Jerrold Agbankpe, A., Hounsa, E., Vodounnon K., Mousse, W., Ahouandjinou, S., Hinnilo, C., Togbe, E., Kelome, N., Agbangla, C., Sourou Bankole, H., Baba-Moussa, L., & Unuabonah, I.E. (2023). National Survey of Stream Water Quality Revealing Threats to Antibio-Resistant Bacteria, Antibiotic Residues and Heavy Metals in Benin. *Pollution*, 9 (2), 678-692. http://doi.org10.22059/POLL.2022.348629.1629

DOI: http://doi.org10.22059/POLL.2022.348629.1629

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INTRODUCTION

For several decades, streams have played an important role in the development of nations through their importance in agriculture and transportation, without forgetting that they are also the receptacle of municipal wastewater (Kubera, 2021). Thus, they are often exposed to physical, chemical and biological contamination (Tissera et al., 2013). The continual release of antibiotics into the aquatic ecosystem is due to their uncontrolled and anarchic use (Tshibanda et al., 2021). The main origin of these substances in natural waters being runoff, livestock and effluent discharges from wastewater treatment plants (Zhang et al.,2020). Thus, reservoirs of multidrug resistant bacteria and resistance genes are formed through the interaction between anthropogenic effluents (hospital effluents, wastewater from slaughterhouses, industries or communities) and streams (rivers and lakes) (Marti et al.2014). In addition, the presence of antibiotic residues and multidrug resistant bacteria in human and animal feces leads to their introduction into wastewater and then into streams (rivers and/or lakes) through direct discharge or wastewater treatment systems (Zhang et al.,2020). All these interactions promote the growth of multidrug resistant bacteria in streams, through the contact of high bacterial density with high therapeutic concentrations of antibiotics under nutritionally favorable conditions (Rodriguez-Mozaz et al.,2015). In low- and middle-income countries (LMICs) such as Benin, the situation of contamination of streams by antibiotic residues and the development of multi-drug resistant bacteria is still dramatic. This is due to the high level of poverty of the population, the absence of inadequacy of health care facilities and poor sanitation and water treatment infrastructure (Collignon et al., 2018; Croix, 2019). This situation increases the risk of bacterial infection and therapeutic failures in humans due to the consumption of water (drinking) and fish containing antibiotic residues and contaminated by multi-drug resistant bacteria. Apart from the contamination of streams with antibiotic residues and multi-drug resistant bacteria, their pollution with heavy metals is one of the greatest threats to the aquatic ecosystem due to their persistence and possible bioaccumulation and biomagnification in food chains (Jovanović et al.,2017). Heavy metal contamination of the aquatic ecosystem can originate from industrial or community wastewater, pesticide and inorganic fertilizer use, runoff, landfill leaching, marine and port activities, as well as geological alteration of the earth's crust and atmospheric deposition (Ajima et al.,2015). The capacity of bioaccumulation of heavy metals in several aquatic organisms, and thus in fish has been demonstrated by several authors (Verep et al.,2012; Janjić et al.,2015; Jovičić et al., 2015). Similarly, studies have shown that the accumulation of heavy metals in fish at given concentrations, can be considered as indicators of public health problems for human who are fish consumers (Abdel-Baki et al.,2011; Montalvo et al.,2014). The level of bioaccumulation and bioavailability of heavy metals in fish waters and tissues is under the influence of several factors, such as fish biological habitats and physico-chemical parameters of waters (water temperature, pH, oxygen concentration in water, electrical conductivity, turbidity, redox potential and total dissolved solids) (Kehrig et al.,2013; Jovanović et al.,2017). A few studies in Benin have focused on microbial contamination of water bodies (Ichola et al.,2021; Koudokpon et al.,2021), but very few have provided information on the antibiotic residues and heavy metal. In order to prevent the health of aquatic environment, of animals and of Beninese's, it is important to evaluate the level of contamination of the main streams of Benin with multidrug resistant bacteria, antibiotic residues, heavy metals and to understand the influence of physicochemical factors of the water on the bioaccumulation of heavy metals. It is in this same context that the present study was initiated. Its objectives are to identify multidrug resistant bacteria, to detect and quantify antibiotic residues, to measure heavy metals and to measure the physicochemical parameters of the samples of Benin's streams.

MATERIALS AND METHODS

Stream surface water samples were collected from all over Benin (Ganvié, Grand-Popo, Bopa, Tori, Porto-Novo, Adjohoun, Tokpa, Zangnannando, Kota, Tanougou, Koumagou, Malanville, Okpara, Sota, Mékrou, Pendjari) (Figure 1) from 16th April to 28th June 2021. The samples were collected aseptically in 1L bottles and stored in a cooler with ice packs and processed in a laboratory within 24 hours. At each stream surface, 10 points were randomly selected and samples were taken at these points (Table 1). Thus, 10 stream surface water samples were collected at each stream surface. In total, of 160 stream surface water samples were collected and analyzed.

After homogenization, 600 ml of each sample was filtered twice at a rate of 300 ml per filtration. The filtrations were performed on membranes with a pore size of 0.20 µm. For each sample, the two membranes were incubated on eosin methylene blue (EMB) and Chapman agar plates, respectively. Then, the plates were incubated at 37°C for 24 hours. These both culture media were respectively used for the isolation of colonies of enterobacteria (Gram negative bacilli) and cocci. Two or three characteristic colonies obtained on the both culture media used were selected. These colonies were streaked on Mueller-Hinton agar plates for purification and then Gram staining, biochemical tests (catalase and oxidase), seeding of the API 20E gallery (for only Gram-negative bacilli), and free staphylocoagulase and DNAse tests (for only Gram-positive cocci) were performed for the identification of Enterobacteriaceae and *Staphylococcus aureus* strains (Ichola et al.,2021).

All identified bacteria were subjected to antimicrobial susceptibility testing on Muller-

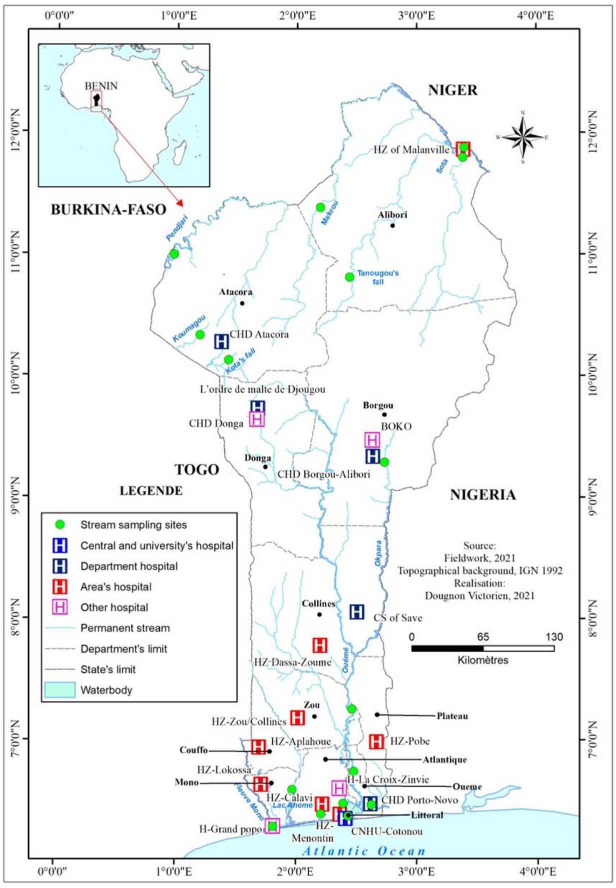

Fig. 1. Sample collection sites

Table 1. Summary of stream samples by region and location of origin and stream type

Benin regions	Location or name of the streams	Types of streams	Number of samples
Northern	Kota	waterfalls	10
	Tanougou	waterfalls	10
	Koumagou	River	10
	Malanville	River	10
	Okpara	River	10
	Sota	River	10
	Mékrou	River	10
	Pendjari	River	10
Southern	Ganvié	River	10
	Grand-Popo	River	10
	Bopa	Lake	10
	Tori	River	10
	Porto-Novo	River	10
	Adjohoun	Lake	10
	Tokpa	Lake	10
	Zangnannando	Lake	10
	Total		**160**

Hinton agar plates using the Kirby–Bauer disc diffusion method to the following antibiotics: ciprofloxacin (5 μg), chloramphenicol (30 μg), metronidazole (5 μg), aztreonam (30 μg), ceftriaxone (30 μg), ertapenem (10 μg), imipenem (10 μg), amoxicillin + clavulanic acid (30 μg), gentamicin (10 μg) and nalidixic acid (30 μg). Measured inhibition zone diameters were interpreted according to EUCAST guidelines (EUCAST,2019). The reference strain *Escherichia coli* ATCC 25922 was used as a control.

Seven antibiotics were searched in this study. These were amoxicillin (AMO) and ampicillin (AP) of the β-lactam family, chloramphenicol (CAP) a phenicol, ciprofloxacin (CPFX) a fluoroquinolone, metronidazole (MNZ) an imidazole, sulfamethoxazole (SMZ) a sulfonylurea and neomycin (NEO) an aminoglycoside. They were chosen in view of their frequency of use in hospitals in West African countries (Dougnon et al.,2020). Both the detection and the quantification were carried out according to the instructions of the MyBioSource® ELISA kit specific to each antibiotic sought. The plate in each kit was read at 450nm on a microplate reader and then the calibration curve of the standards was plotted with the percentages of absorbances (A/A0x100 with A: average absorbance of the standard or sample and A_0 the average absorbance of the 0ppb standard) as a function of logC of the standards contained in each kit. The determination of sample concentrations from the linear equation (y=ax+b) of the calibration curves (Figures 2 and 3) obtained for each antibiotic was performed.

The determination of five metals, namely mercury, lead, zinc, cadmium and nickel, was carried out by the method of the MERCK test kits specific to each metal. The concentrations were determined using a photo Lab 6600UV-VIS molecular absorption spectrophotometer. The principle is that in the presence of (pyridyl-2'-azo)-4-resorcinol (PAR), the metal forms a complex which is determined photometrically. The concentrations of mercury, zinc, cadmium and nickel are directly determined with the Photo Lab 6600UV-VIS. For lead, the process also depends on the purity of the water and requires a total hardness test. With the hardness determination, the lead analysis is done in two steps. A first reading with a molecular absorption spectrophotometer after addition of reagent A and a second one after addition of reagent B. The

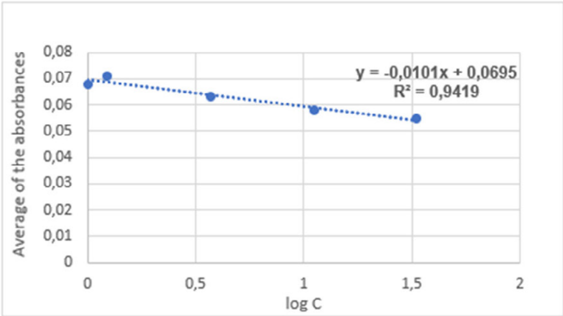

Figure 2a. Amoxicillin calibration's curve

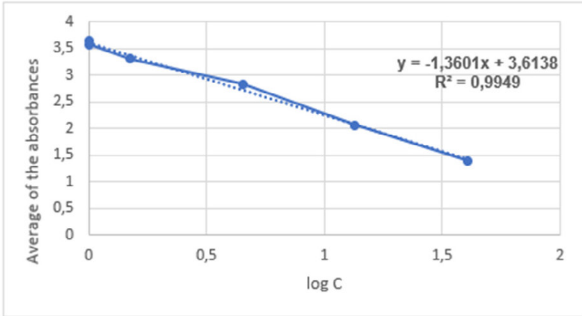

Figure 2b. Ampicillin calibration's curve

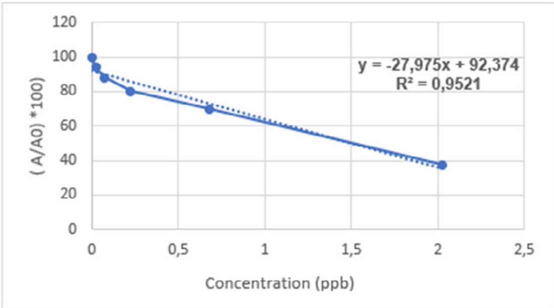

Figure 2c. Chloramphenicol calibration's curve

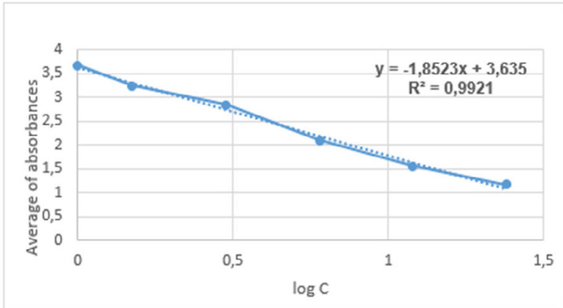

Figure 2d. Metronidazole calibration's curve

Fig. 2. Calibration curves for Amoxicillin, Ampicillin, Chloramphenicol and Metronidazole

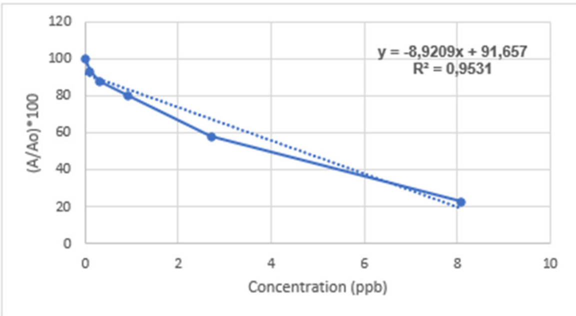

Figure 3a. Ciprofloxacin calibration's curve

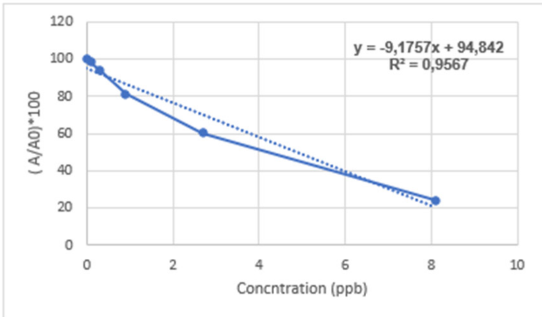

Figure 3b. Sulfamethoxazole calibration's curve

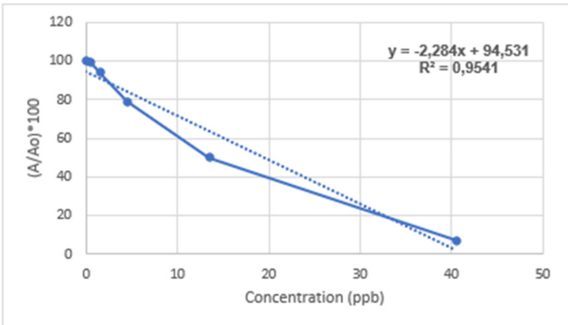

Figure 3c. Neomycin calibration's curve

Fig. 3. Calibration curves for Ciprofloxacin, Sulfamethoxazole and Neomycin

final lead concentration in the sample is the difference between the values obtained after the different readings.

The physicochemical characterization was performed to determine the following parameters: temperature, hydrogen potential (pH), salinity, electrical conductivity (EC), resistivity, redox potential (P-redox) and total dissolved solids (TDS). They were analyzed according to the protocols recommended by (Rodier et al.,2009) by direct measurement with a multimeter Multi 3630 IDS SET KS2.

Data were recorded in Excel 2016 spreadsheet; the same software was used to perform the different antibiotic calibration curves. R software was used for data analysis. Two-way ANOVA test was used for multiple comparison between the mean concentrations of antibiotic residues, values of physicochemical parameters and concentrations of heavy metals of the river samples from Northern and Southern Benin. The graphs were produced using GraphPad Prism 7 software.

RESULTS AND DISCUSSION

Rivers are dynamic, ever-changing ecosystems that undergo many changes due to natural processes and human activity. Anthropo-pression has a significant impact on water quality in aquatic environments and on human health (Gotkowska-Plachta et al.,2015). Thus, a characterization of multi-drug resistant bacteria and antibiotic residues, an assessment of heavy metal contamination of streams and an understanding of the relationship between physicochemical water parameters, heavy metals and multi-drug resistant bacteria would be important and urgent in order to reduce the risks of infections and heavy metal contamination. This is what this study was dedicated to. From the 160 surface water samples analyzed, we isolated 283 bacterial strains of which 64.31% (n = 182) were identified to the species level and 35.69% (n = 101) were identified as non-enterobacteria. Of One hundred eighty-two bacterial species identified, *Klebsiella pneumoniae* was the most represented species with 56.59% (n = 103), followed by *Klebsiella* spp. (18.68%; n = 34) and *Enterobacter* spp. (12.63%; n = 23) (Figure 4).

We've observed an abundance of non-enterobacterial and *Klebsiella pneumoniae* strains but also the presence of other bacterial species such as *E. coli*, *Enterobacter* spp, *Salmonella* spp and *Klebsiella* spp. In Benin, Ichola et al. (2021) also observed an abundance of the genus *Klebsiella* and the presence of non-enterobacteria, *E. coli* species, and *Enterobacter* spp in water samples from the Cotonou-Lac Nokoué hydrographic complex. Studies in Algeria and Nigeria have

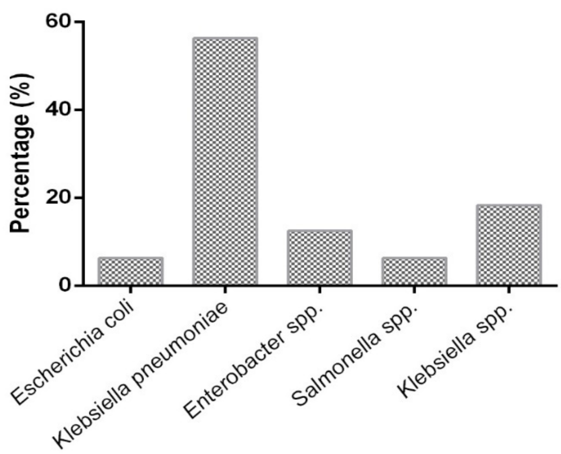

Fig. 4. Percentage of different bacteria species identified

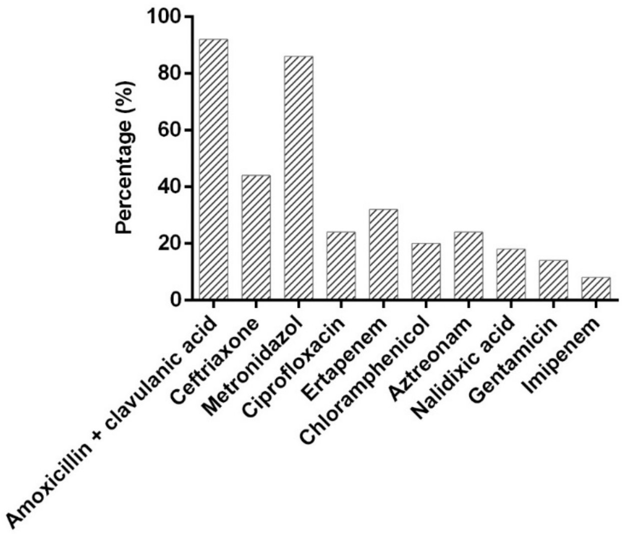

Fig. 5. Percentage of resistance of bacterial strains to antibiotics used

reported presence of non-enterobacteriacea, and *Klebsiella* spp in water samples as was detected also in our study (Olaoye et al.,2009; Djenadi et al.,2018). The same species (resistant) were also found in some wastewater samples collected in Seme Krake, a town in southern Benin (Koudokpon et al.,2021).

Ten different antibiotics belonging to several different families were used in order to determine the resistance level of the identified bacterial isolates. The most abundant resistance of the bacterial strains was to amoxicillin + clavulanic acid (92%), followed by metronidazole (86%), ceftriaxone (44%), ertapenem (32%), ciprofloxacin (24%), and aztreonam (24%) (Figure 5). It should be noted that all bacterial strains are resistant to more than three antibiotics belonging to different families, thus multidrug-resistant.

The bacterial strains isolated from the stream samples were all multidrug resistant with a low resistance to phenicol, aminoglycosides and more especially fluoroquinolones has been observed. Sani et al.,2018 have also obtained similar results. A strong resistance to beta-lactams and imidazole has been recorded. These results are similar to those of (Azaiez et al.,2019 ; Khalfallah et al.,2021) who have reported a resistance to beta-lactams. The resistance of strains of enterobacteria and non-enterobacteria to beta-lactams is an emergence reported by the World Health Organization (WHO,2017), as priority resistance in human health. This shows that the rivers of Benin constitute a reservoir of pathogens indicators of risk to public health. Resistance to imidazole, more precisely metronidazole observed in our study has also been shown by some studies (Gombima,2021). Our result is different from those obtained by (Azaiez et al.,2019) in which strains were sensible to metronidazole. This resistance could be explained by the fact that this antibiotic is considered as a dewormed and an antispasmodic by the populations of Benin and taken without control to treat bacterial infections (Ichola et al.,2021).

The detection of antibiotic residues in the surface water of the 16 collected streams showed that all the searched antibiotics were detected, except for sulfamethoxazole and neomycin. Chloramphenicol and metronidazole were detected in all streams sampled. Amoxicillin was detected in 4 streams, 2 in the north (12.5%) and 2 in the south (12.5%). As for ampicillin residues, they were detected in 8 rivers in the south (50%) and in 4 in the north (25%). Finally, ciprofloxacin residues were detected in 50% (6/16) and 37.5% (8/16) of the streams sampled in the south and north, respectively (Table 2).

The concentrations of antibiotic residues varied from one course sample to another.

Table 2. Distribution of samples according to the frequencies of detection of antibiotic residues

Antibiotics detected	Streams samples (n = 32)			
	Streams from Northern (n = 16)		Streams from Southern (n = 16)	
Amoxicillin (AMO)	2	(12.5 %)	2	(12.5%)
Ampicillin (AP)	4	(25%)	8	(50%)
Chloramphenicol (CAP)	16	(100%)	16	(100%)
Metronidazole (MNZ)	16	(100%)	16	(100%)
Ciprofloxacin (CPFX)	6	(37.5%)	8	(50%)
Sulfamethoxazole (SMZ)	-	-	-	-
Neomycin (NEO)	-	-	-	-

Table 3. Mean concentration of antibiotic residues according to the two regions of Benin where the streams were collected

Antibiotics residues	Mean concentrations of antibiotic residues detected (µg/L)	
	Northern	Southern
Amoxicillin (AMO)	0.111 ± 0.016	0.065 ± 0.005
Ampicillin (AP)	1.550 ± 0.050****	2.415 ± 0.065****
Chloramphenicol (CAP)	0.104 ± 0.004	0.106 ± 0.003
Ciprofloxacin (CPFX)	1.395 ± 0.045****	3.010 ± 0.003****
Metronidazole (MNZ)	6.635 ± 0.035	6.700 ± 0.050

****: p-value < 0.0001

Metronidazole was the antibiotic with the highest residue concentration in the samples (6.578 to 6.829 µg/L), followed by ciprofloxacin with residue concentrations ranging from 2.142 to 9.299 µg/L and ampicillin (1.272 to 4.235 µg/L). The comparison of the average concentrations of the different antibiotic residues obtained in the northern and southern streams showed that the concentrations of chloramphenicol and metronidazole residues in the southern streams (0.106 ± 0.003 µg/L; 6.700 ± 0.050 µg/L) are higher than those obtained in the northern streams (0.104 ± 0.004 µg/L; 6.635 ± 0.035 µg/L). In contrast, the concentration of amoxicillin residues in northern streams (0.111 ± 0.016 µg/L) is higher than those in southern streams (0.065 ± 0.005 µg/L). However, the difference between these different antibiotic residue concentrations is not statistically significant. On the other hand, there is a statistically significant difference between the mean concentrations of ampicillin and ciprofloxacin residues obtained in the northern and southern streams of Benin, with higher concentrations of these two antibiotics in the southern (2.415 ± 0.065 µg/L; 3.010 ± 0.003 µg/L) streams than in the northern (1.550 ± 0.050 µg/L; 1.395 ± 0.045 µg/L) one ($p < 0.0001$) (Table 3).

Various antibiotic residues have been detected in stream surface water samples from Benin. Aquatic organisms are thus exposed to cocktails of antibiotics. Even if their individual concentrations measured in the environment are low, when combined, they can be significant and cause significant toxicity to aquatic organisms (Everaert et al.,2020) by interacting with each other having either a large effect (synergistic effect) or a smaller effect together than alone (antagonistic effect) (Danner et al.,2019). Metronidazole, ciprofloxacin and ampicillin were also detected at high concentrations in the rivers of northern and southern Benin. This could be explained by the frequent use of these molecules in human and animal health but also by their low biodegradation in the environment. The concentrations found in the environment are highly variable in relation to variations in consumption patterns (type of antibiotic and quantity) and with the persistence of the different compounds disseminated (Peralta et al.,2016). On the other hand, the conditions of the receiving environments and the intrinsic chemical characteristics of the molecules condition their persistence (Michael et al.,2013). Therefore, the study of processes governing persistence such as sorption, mobility and degradation of antibiotics is an essential step. The most important mechanisms conditioning sorption and thus mobility of antibiotic compounds are interactions with organic matter and mineral constituents in the soil, as well as ion exchange, hydrogen bonding and complex formation with metal ions (Morel et al.,2014). In addition, the lack of sanitation and wastewater treatment facilities in low-income countries means that the average concentration of antibiotic residues is generally much higher.

The concentration of heavy metals varies from one sample to another. The average concentration of zinc (6.126 ± 16.017 mg/L) was the highest of the other metals tested in southern Benin streams, followed by mercury (0.454 ± 0.129 mg/L), cadmium (0.140 ± 0.159 mg/L) and nickel (0.126 ± 0.163 mg/L). In terms of heavy metal concentrations in the northern streams, mercury (0.277 ± 0.147 mg/L) was the highest, followed by nickel (0.155 ± 0.233 mg/L), cadmium (0.154 ± 0.132 mg/L) and zinc (0.139 ± 0.114 mg/L). The difference between the average concentrations of heavy metals in the northern and southern Benin streams is not statistically significant (Table 4).

All the metals searched for were found in concentrations largely superior to the standards tolerated in Benin with a predominance of zinc (Zn). These results highlight the environmental danger and the health risks to which the surrounding populations are exposed when we know that in Benin, these waterways represent a source of water supply in certain localities. Various studies including those of (Dimon et al.,2014; Hounkpè et al.,2017) have also reported high concentrations and above the standards illustrating the toxicological pollution of Beninese waterways. Anthropogenic activities as well as runoff water constitute a non-negligible source of pollution of this aquatic ecosystem (Hounkpè et al.,2017)

The values of the different physicochemical parameters vary from one river to another. The conductivity of Benin's rivers varies from 7.55 to 22300 µS/cm. The mean conductivity of the southern streams (8770.081 ± 12611.220 µS/cm) was significantly higher than that of the northern streams (111.237 ± 51.205 µS/cm) ($p = 0.0073$) (Table 5). Total dissolved salts (TDS) had the same values as conductivity in each stream. Like conductivity, its mean value in the southern streams (8770.081 ± 12611.220 mg/L) is significantly higher than in the northern streams (111.237 ± 51.205 mg/L) (table 5). Temperature is one of the essential parameters governing chemical equilibrium and the development of flora and fauna in our waterways.

The average temperature of the rivers in southern Benin was 26.75±0.434°C with a maximum temperature of 27.3°C and 26.4°C as minimum temperature. The average temperature of the northern rivers was 27.237±0.292°C with 27.5°C and 26.6°C as the maximum and minimum temperature respectively (table 5). The pH is a measure of the acidity of the water, i.e. the concentration of hydrogen ions (H^+). The pH of Benin's rivers varies from 5.73 to 7.67. Streams in the south have a mean pH of 6.604±0.658. With a slight difference, the mean pH of the northern streams was 6.579±0.256 (table 5). Our results showed that the salinity of rivers in northern Benin was 0%. Similarly, of the 8 streams sampled in southern Benin, 4 had zero salinity. The salinity of the other 4 southern streams varied from 4.2 to 19.2% with a mean of 5.825±7.475% (table 5). The mean of redox potential of the rivers in northern and southern Benin was 21.438±39.048 mV and 22.938±11.193 mV, respectively. It should be noted that the redox potential of three rivers in the south was negative (Ganvié river, Grand-Popo river and lake Ahémé) as well as the Sota river in the northern region of Benin (table 5). The mean resistibility of streams in southern Benin was 6.490±9.008 KΩ/cm. While that of the northern streams was 15.225±6.238 KΩ/cm. However, this difference in stream resistibility between these two regions

Table 4. Concentrations of heavy metals in streams by region and sampling location in Benin

Streams	Heavy metal concentration in streams samples					
	Suspended solids (mg/L)	Mercury (µg/L)	Lead (mg/L)	Zinc mg/L	Nickel (mg/L)	Cadmium (mg/L)
Southern						
Ganvié river	0.09	0.5076	0.13	0.229	-	0.033
Grand-Popo river	0.02	0.6046	-	0.014	-	0.143
Lake ahémé (Bopa)	0.12	0.6632	0.03	0.109	0.23	0.073
Tori river	0.02	0.4272	-	-	0.07	0.125
Porto-Novo river	0.04	0.4098	0.08	0.148	-	0.04
Lake tovègbamé (Adjohoun)	0.06	0.3094	-	0.096	0.4	0.106
Lake nokoué (Tokpa)	0.19	0.4036	0.08	45.7	0.31	0.075
Lake azili (Zangnannando)	0.5	0.3065	0.0	2.71	-	0.522
Mean ± SD	**0.130 ± 0.160[a]**	**0.454 ± 0.129[b]**	**0.040 ± 0.050[c]**	**6.126 ± 16.017[d]**	**0.126 ± 0.163[e]**	**0.140 ± 0.159[f]**
Northern						
Falls of kota	0.02	0.3365	-	-	-	0.06
Falls of tanougou	0.32	0.1822	-	0.043	0.05	0.032
Koumagou river	0.04	0.6204	0.11	0.091	-	0.055
Niger river	0.27	0.2337	0.0	0.213	0.46	0.335
Okpara river	0.13	0.2138	-	0.226	0.58	0.182
Sota river	0.02	0.2188	-	0.156	0.15	0.137
Mekrou river	0.06	0.1924	0.0	0.336	-	0.369
Pendjari river	0.02	0.1917	-	0.051	-	0.061
Mean ± SD	**0.110 ± 0.121[a]**	**0.277 ± 0.147[b]**	**0.014 ± 0.039[c]**	**0.139 ± 0.114[d]**	**0.155 ± 0.233[e]**	**0.154 ± 0.132[f]**

of the country is not statistically significant (table 5).

The analysis of the physicochemical quality of the streams through some parameters revealed that these last ones vary in space. Apart from the electrical conductivity, all other parameters are in conformity with the standards. The mean concentrations of suspended solids (SS) are all in compliance with WHO and Beninese standards respectively (20mg/l and 35mg/l). Indeed, as a result of anthropogenic activities, chemicals released into the environment can enter aquatic ecosystems and become incorporated into suspended solids (Dimon et al.,2014). Their presence in groundwater prevents light penetration and poses a risk to fish species in that it causes their death through gill plugging (Dimon et al.,2014). Electrical conductivity, resistivity and total dissolved solids (TDS) are parameters that allow us to assess the overall mineralization of a water. Thus, a low conductivity for a stream is also synonymous with a low mineralization of salts present in the environment (Ben Moussa et al.,2012). External inputs of water into the aquatic environment contributes to the dilution of these parameters with the corollary of low values of these parameters (Zinsou et al.,2016). In the rivers, only those in the South showed a mean conductivity (87700.81µS/cm) very much higher than the norm with high levels in the So (22300µS/cm) and Ouémé (16440µS/cm) rivers as well as in Lake Nokoué (31000µS/cm). The

Table 5. Values of physicochemical parameters of streams samples

Streams samples	Physicochemical parameters						
	Conductivity (µS/cm)	**Temperature (° C)**	**pH**	**Salinity (%)**	**Total dissolved salts (mg/L)**	**Redox potential (mV)**	**Resistibility (KΩ/cm)**
Southern							
Ganvié river	22300	27.2	6.99	13.5	22300	-1.2	0.0448
Grand-Popo river	148.2	26.6	6.98	0	148.2	-0.9	6.74
Lake ahémé (Bopa)	7.55	26.4	7.67	4.2	7.55	-41.9	0.1322
Tori river	144.8	26.4	6.33	0	144.8	37.7	6.91
Porto-Novo river	16440	27.3	6.45	9.7	16440	30.6	0.0608
Lake tovègbamé (Adjohoun)	81.2	27.3	5.73	0	81.2	73.1	12.3
Lake nokoué (tokpa)	31000	26.4	6.89	19.2	31000	4.3	0.0323
Lake azili (zangnannando)	38.9	26.4	5.79	0	38.9	69.8	25.70
Mean±SD	**8770.081 ± 12611.220****	**26.75 ± 0.434**	**6.604 ± 0.658**	**5.825 ± 7.475**	**8770.081 ± 12611.220****	**21.438 ± 39.048**	**6.490 ± 9.008**
Northern							
Falls kota	89.8	27.4	6.75	0	89.8	12.7	11.16
Falls tanougou	185.2	27.5	6.37	0	185.2	35.4	5.4
Koumagou river	40.5	27.2	6.25	0	40.5	42.6	24.7
Niger river	114.7	27.1	6.4	0	114.7	33.4	8.73
Okpara river	120	27.3	6.4	0	120	33.5	8.33
Sota river	182.4	27.3	6.98	0	182.4	-0.8	5.48
Mékrou river	87.1	27.5	6.77	0	87.1	11.4	11.48
Pendjari river	70.2	26.6	6.71	0	70.2	15.3	14.26
Mean±SD	**111.237 ± 51.205****	**27.237 ± 0.292**	**6.579 ± 0.256**	**0**	**111.237 ± 51.205****	**22.938 ± 11.193**	**15.225 ± 6.238**

**: $p < 0.01$

rivers in the South have a mean resistivity of 6.49 KΩ/cm. On the other hand, the So, Ouémé and Nokoué streams showed significant mineralization. The high TDS contents of these three streams reflect their character translate the presence of salts, minerals and metals. This result is corroborated by the mean salinity (5.82%). In sum, the rivers studied in the South, notably the So River, Ouémé and Lake Nokoué, have high mineralization, are loaded with total dissolved solids and therefore have a high salt content and are therefore unpleasant waters. On the other hand, in the North, the mean values of conductivity, total dissolved solids and resistivity allow us to conclude that they are weakly mineralized with a low or zero content of salt (0%), minerals and metals.

CONCLUSION

The analysis of streams in northern and southern Benin revealed the presence of antibiotic residues in significant concentrations and of multi-drug resistant bacteria. The results show that the concentrations of antibiotic residues obtained are likely to have direct or indirect effects not only on the microbial component of aquatic populations but also on human health. This situation must lead us to evaluate the risk presented by these molecules but also to improve techniques and develop methods adapted to limit the release of these residues into the environment because they also participate in the increase of the antibiotic resistance phenomenon. Our study showed the presence of several heavy metals (mercury, lead, zinc, nickel and cadmium) in Benin's streams. The sources of the presence of these metals in the streams are domestic and industrial wastes and the excessive use of phosphatic fertilizers. Apart from lead, the average concentration of mercury, zinc, nickel and cadmium was above the norm in Benin. This justifies that there is a risk of heavy metal contamination in aquatic organisms and in humans who consume water and fish.

ACKNOWLEDGEMENTS

The authors are very grateful to The World Academy of Sciences (TWAS) and the Islamic Development Bank (IsDB), which funded one of them for this study. They are finally grateful to the Minister of the Living Environment and Sustainable Development in Benin, his Excellency his Excellency M. Jose´ TONATO and all his staff.

AUTHORS' CONTRIBUTIONS

EG, MCHQ, TVD, AO, NK, CA and HB wrote the protocol. WM and IEU got the funding. KF, AJA, EH, KV, SA, CH and ET processed the samples. TVD, JRK performed the statistical analyses. TVD, AJA and JRK wrote the draft of the manuscript. All authors read and approved the final manuscript.

GRANT SUPPORT DETAILS

The authors are very grateful to The World Academy of Sciences (TWAS) and the Islamic Development Bank (IsDB). These two institutions have made this research possible through research funding allocated to the research team under the IsDB-TWAS Grants for Research Collaboration in Sustainability Sciences (506808). They reviewed the research protocol and validated the design of the study and collection, analysis, and interpretation of data.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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