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Research Paper

Dynamic of antibiotic resistant enteric bacteria in the lakeObili-Yaoundé(Cameroon)

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ABSTRACT:*A study was conducted to assessthe dynamic of antibiotic resistant enteric bacteria in the lake Obili-Yaoundé (Cameroon). Water samples were collected at three different points of the lake (the entrance, the middle and the exit) for microbiology analysis. A total of 21 isolates of enteric bacteria were pre-identified and presented higher resistance rates to antibiotics of the β-lactam family [(AMX, 100 %), (CAZ, 71,4 %) and (CTX, 57,1 %)], followed by tetracyclines (TET, 76,2 %), the aminosides (KAN,61,9 %),and finally the fuoroquinolones (CIP,52,4%). The 21 pre-identified isolates were identified as belonging to the following species Escherichia coli (07), Proteus vulgaris (03), Enterobacter aerogenes (02), Proteus rettgeri (02), Salmonella spp (02), Enterobacter agglomerans (01), Salmonella paratyphi (01), Salmonella typhi (01), Klebsiella spp (01) and Citrobacter spp (01). In conclusion, the lake Obili is a source of an intense fecal contamination, harbors a diverse community of drug resistant enteric bacteria, and represents a vector for dissemination of antibiotic resistance.*

KEYWORDS:*Antibiotic resistance, enteric bacteria, third generation cephalosporins,lake Obili.*

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I. INTRODUCTION

Surface waters are commonly used in agriculture, animal husbandry, food for populations and industry. However, surface waters receive daily domestic, agricultural and industrial discharges and are consequently transformed into sites of concentration of both chemical and biological pollution [1]. Enteric bacteria are Gram negative bacilli, ubiquitous, and are used as witness of fecal contamination. Their abundance in the intestine, their mobility, the speed of their multiplication, the frequent acquisition of resistance mechanisms to antibiotics explain why these bacteria are most involved in human infectious pathologies, especially in hospitals [2]. Most of antibiotics used in humans and animals' treatment are only partially metabolized after their administration, thus, these are excreted in urine and feces, and discharged into various compartment of environment [3].

The lake Obili is a large surface water retention pond on the Olezoa river, located in Yaoundé- Cameroon. The water of the lake Obili, which areof interest for our study, are intended for multiple uses. The lake Obili is an ecosystem presenting an important biological activity with a diverse fauna and flora that it shelterswhich gives it a conservation role (reproduction, development), and offers a favorable environment for scientists and research on various issues related to aquatic environments. Fish farming and agriculture which is the primary sector of water consumption, especially for crop irrigation is practiced. The waters of lake Obili are not spared by organic pollution of anthropogenic origin, due to strong colonization of the watershed by human, animal and plant, the abusive use of water body as an outlet for waste and septic tank [4]. The aim of this study was to assess the dynamic of antibiotic resistant enteric bacteria in the lake Obili-Yaoundé.

II. MATERIAL AND METHODS

Study area and sampling strategy

The lake Obili islocated in the town of Yaoundé, in the Center region of Cameroon. The lake Obili was created in 1948 by the Ministry of water and forests. Many activities are practices in lake Obili such as the supply of fry for fish farmers, the popularization of breeding methods, agriculture, and serves as a laboratory for studying aquatic ecosystems in tropical zone [5]. Five samplings were conducted over three months (February, April and

June 2021), at three points (the entrance, the middle and the exit) of the lake. At each sampling point, three samples were taken, resulting in a total of nine samples per sampling. The samples were collected by immersing a sterile vial at 15 cm below the water, the opening of the vials facing the opposite direction of the water flow to avoid collection of particles deposited on the surface as well as those from sediments. After collection, the samples were placed in a cooler and transported at 4°C to the laboratory for analysis [6].

Isolation, enumeration andmacroscopicobservation

Water samples (1 ml each) were introduced in test tubes previously containing 9 ml of sterile water to make several 10-fold serial dilutions up to the 10^{-6} dilution. From stock solutions and 10^{-1}, 10^{-2} and 10^{-3} dilutionswere prepared, and 0.1 ml was spread on MacConkey agar and incubated at 37°C for 24 h. after incubation, macroscopic observation of the colonies was performed allowing a first characterization with possible orientation of results during the identification. The macroscopic identification elements were: the shape of the colonies (round, irregular, etc.), the size of the colonies (by measuring the diameter), the color of the colonies, the elevation (concave, convex, flat, domed), opacity (opaque, translucent or transparent) and surface (smooth, rough, dry, jagged... etc.)[7].

Antimicrobial susceptibility testing

To assess the phenotypic resistance of the different isolates, antimicrobial susceptibility testingwas conducted by disk diffusion method on Mueller-Hinton agar, according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [8]. Six antibiotics were used: Amoxicillin (AMX, 25 µg), Ceftazidime (CAZ, 30 µg), Cefotaxime (CTX, 30 µg), Kanamycin (KAN, 30 µg), Tetracycline (TET, 30µg) and Ciprofloxacin (CIP, 5 µg). A bacterial suspension in physiological water was prepared and adjusted to a 0.5 McFarland standard. Using a micropipette, 0.1 ml of the bacterial suspension was spread on Mueller-Hinton agar with a sterile inoculator. The antimicrobial disks were dispensed with automatic disk dispensers and plates were inoculated for 24h at 37°C. The grown isolates were scored as susceptible, intermediate or resistant to a given antibiotic by the inhibition zone diameter around the disk and according to the CA-SFM recommendations [8].

Biochemical identificationand microscopic observation

Several biochemical tests to assess the metabolic activities of the different isolates for identification were performed such as oxidase test, catalase test, research into use of glucose, lactose, gas and H_2S production on Kliger-Hajna (KIA) medium, study of mannitol degradation and mobility on Mannitol-Mobility medium and urease test on Urea-Indole medium [9].Thereafter, microscopic observation was conducted after Gram staining.

Data analysis

The data obtained were analyzed using Microsoft Excel 2016 spreadsheet. The mean values of the abundance of enteric bacteria obtained during the different sampling periods were compared by analysis of variance (ANOVA), followed by Tukey's post hoc test at$p \leq 5$ %, using SPSS 16 software.

III. RESULTS AND DISCUSSION

The mean values of enteric bacteria abundanceobtained during the different sampling period are reported in Table 1.

Table 1. Abundance of enteric bacteria (CFU/ml)during the sampling periods.

Periods	Sampling areas		
	Entrance	Middle	Exit
10/02/2021	249	180	318
05/04/2021	200,3	180	298,6
26/04/2021	290,8	180,15	322,35
01/06/2021	230,4	199,8	245,6
21/06/2021	255	178	386
Mean values in CFU/ml (± SD)	1226 UFC/ml (± 33,3)^{b}	918 UFC/ml (± 9,1)^{c}	1571 UFC/ml (± 50,5)^{a}

Values followed by the same letters are not significantly different Tukey's test($P<0.05$)

At all sampling points, the enteric bacteria abundanceexceeds the threshold set by the World Health Organization (WHO) for a direct discharge of wastewater into environment (10^3 CFU/100 ml); similarly, these

concentrations exceed at all points the level recommended by the WHO for the use of water intended for irrigation of crops (10^3 CFU/100 ml) [10].Therefore, these waters are improper for any use for human activities.
Intermediate and resistant isolates were subsequently grouped in same resistant class to calculated the resistant percentages
The percentage of resistance of the different isolates to the tested antibiotics is reported in Table 2.

Table 2. Percentage of resistance of the different isolates to the tested antibiotics

Antibiotics (ATBs)	Number of resistant isolates	Number of intermediate isolates	Percentage of resistance
Amoxicillin (AMX)	20 isolates	1 isolate	100 %
Ceftazidime (CAZ)	4 isolates	11 isolates	71,4 %
Cefotaxime (CTX)	10 isolates	2 isolates	57,1 %
Kanamycin (KAN)	7 isolates	6 isolates	61,9 %
Tetracycline (TET)	13 isolates	3 isolates	76,2 %
Ciprofloxacin (CIP)	6 isolates	5 isolates	52,4 %

The enteric bacteria isolates showed a resistant rate of 100 % to amoxicillin. This result is in accordance with that of [11], who in a study on the mechanisms of resistance in *Enterobacteriaceae* towards beta-lactams antibiotics, revealed a resistant rate of 99 % to amoxicillin. This high resistance rate of enteric bacteria to amoxicillin is due to the fact that amoxicillin is the most frequently molecule prescribed and consumed in all types of infections from the most benign to the severe in the country. The increase in its consumption over the last 10 years is particularly worrying (+ 30.6 % in human medicine in cities) and goes against all the health policies implemented [12].
The different isolates presented a resistance rate of 76.2 % to tetracycline. These results aredifferent with those of [13], who studied characteristics of extended-spectrum β-lactamase and carbapenemase-producing *Enterobacteriaceae* isolates from rivers and lakes in Switzerland, and reported a resistant rate of 57.6 % to tetracycline. This high resistance rate to tetracycline could be explained by the fact that tetracycline is considered as the antibiotic of choice in pneumonia treatment. Tetracycline resistance is often plasmid in enteric bacteria, hence the rapid dissemination of resistant strains in the external environment [14].
Ceftazidime and cefotaxime are third generation cephalosporins (C3G). Enteric bacteria isolates showed resistant rate of 71.4 % and 57.1 % to ceftazidime and cefotaxime respectively. These results are not in accordance with those of [15], who in a study on antibiotic resistant of Gram-negative bacteria in rivers in United States, reported very low rates of resistant to ceftazidime and cefotaxime of 6 % and 12 % respectively. These high levels of resistant rate to C3G could be explained by the production of enzymes such as extended spectrum β-lactamases (ESBLs) and cephalosporins. C3G are obtained by hemi-synthesis from natural molecules and are considered essential antibiotics and often of last resort for treatment of severe infections caused by Gram negative bacilli [16]. In the contrary to high income countries, tetracyclines, C3G, and other antibiotics are accessible to the public without medical prescription, their consumption is uncontrolled. Its particular alarming to find C3G resistant enteric bacteria in these waters. These represent antibiotics of last resort, and public health systems will be without solution in case of infection by these drug resistant strains.
The isolates presented a resistance rate of 61.9 % to kanamycin. This result is in accordance to that of [17], who on a study on the antibiotic susceptibility profile of bacterial isolates from some fishponds in Yaoundé, reported a resistance rate of 61.9 % to Kanamycin. Kanamycin is an antibiotic which resistance is more frequently encountered in clinic. This high rate obtained in the aquatic environment could be explained by the consequence of the increased consumption of the population, intensive breeding using antibiotics and lack of waste recycling near hospitals. As resistance to kanamycin is often plasmid in enteric bacteria, this made its diffusion more probable by horizontal transfers [18].
Ciprofloxacin was the most effective antibiotic against enteric bacteria isolates which presented a resistance rate of 52.4 %. This rate is low compared to that of [19], whoin a study on antibiotic resistance *Enterobacteriaceae* from surface waters in the urban Brazil highlights the risks of poor sanitation, reported a resistance rate of 82.4 % to ciprofloxacin. This high resistance rate to ciprofloxacin could be explain by the fact that ciprofloxacin is widely used in large number of indications in humans and animals' medicine. It's the antibiotic of whose

consumption has been more decreasing over the last 10 years. Ciprofloxacin is excreted in the urine in unchanged form and is not very biodegradable in the environment [20].
In order to get an idea of the dynamics of antibiotic resistant enteric bacteria present in lake Obili, we have grouped the frequency of different species during the different sampling (Table 3).

Table 3. Frequencyof the different antibiotic resistant enteric bacteria during the different sampling months

	Months		
	February	April	Jun
Enterobacter aerogenes	2	/	/
Entarobacter agglomerans	/	/	1
Klebsiella spp	1	/	/
Proteusrettgeri	/	/	2
Proteus vulgaris	/	2	1
Salmonella paratyphi	/	1	/
Salmonella typhi	/	1	/
Salmonella spp	/	1	1
Escherichia coli	1	2	4
Citrobacter spp	/	1	/

/: *None*

*E. coli*was most prevalent in the lake, and presented 100 % resistance to amoxicillin, 85.7 % resistance to tetracycline, and 42.9 % resistance to cefotaxime, kanamycin, ceftazidime and ciprofloxacine. These rates of resistance are high compared to those reported by [21], who conducteda study on the sensitivity to antibiotics and the presence of the integrons in *E. coli* isolated from an estuary of the Seine (France), and reported low resistance rates of 15.4 %, 3.8 % and 1.2 %, respectively to amoxicillin + clavulanic acid, ceftazidime and cefotaxime; and resistance rates of 43.6 % and 9.6 %, respectively to tetracycline and ciprofloxacin. The high abundance of*E. coli*in the lake Obili could be consequentto a greater fecal contamination since *E. coli* is used as an indicator of fecal contamination.
Proteus vulgaris and *Proteus rettgeri*were 100 % resistant to amoxicillin and presented no resistance to kanamycine. *Proteus vulgaris*was 66.7 % resistant to ceftazidime, cefotaxime, tetracycline and ciprofloxacine. *Proteus rettgeri*was 50 % resistant ceftazidime, cefotaxime, tetracycline and ciprofloxacin. This group of enteric bacteria are common in the digestive tract of humans and animals and can also be found on the skin and mucous membranes. *Proteus* is the second etiology agent responsible ofurinary tract infections (10 % of cases) [22]. The lake Obili could be subject to contamination from hospital wastewaters.
E. coli was isolated during all the sampling times and was more prevalent in the rainy season; *Salmonella* was more prevalent in rainy season and absent in dry season. The presence of *Salmonella* in the waters of the lake Obili constitute a serious public health problem because according to [23], there are an estimated 93.8 million cases of gastroenteritis due to *Salmonella.*These can explain numerous cases of salmonellosis reported from populations living in close proximity with the lake.

IV. CONCLUSION

The data reported present the lake Obili as a source of an intense fecal contamination, and a vector for dissemination of antibiotic resistance. The lake Obili represents a danger for the populations who come into contact with these waters, and consumersof food irrigated with the waters of the lake. Therefore, urgent action is needed for a proper sanitation of the lake and to sensitize the populations of the public health danger that represents the lake.

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