



## Antibiotic susceptibility profile of bacterial isolates from some fishponds in Yaoundé (Cameroon)

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**ABSTRACT :** A study was conducted to assess the antibiotic susceptibility profile of bacteria isolated from some fish ponds in the city of Yaounde (Cameroon). Water samples were collected in fishponds located in five districts of the city of Yaounde (Nkolbisson, Odza, Afan'ayoa, Toutouli, Eto-koss). Bacteria isolated from water samples were resistant to amoxicillin (86%), cefixime (81%), tetracycline (75%), sulfamethoxazole / trimethoprim (65%), kanamycin (62%) and ciprofloxacin (48%). Fifty-six bacterial isolates resistant to antibiotic were isolated. The drug resistant bacteria isolates belonged to following species, namely *Pseudomonas spp*, *Staphylococcus aureus*, *Shigella spp*, *Enterococcus spp*, *Enterobacter spp*, *Escherichia coli*, *Streptococcus spp*, *Salmonella spp*, *Bacillus cereus*, and *Micrococcus spp*. These results showed that fishponds in the city of Yaounde are reservoirs of antibiotic resistant bacteria including those of medical interest, and could represent vectors for the dissemination of antibiotic resistance via direct contact with contaminated water and through the food chain.

**KEYWORDS:** Aquaculture; fishponds; antibiotic resistance; urban agriculture.

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### I. INTRODUCTION

Antibiotic resistance is a growing public health problem concern responsible of about 700,000 deaths annually worldwide. According to the WHO, antibiotic resistance could be the cause of 10 million deaths per year by 2050 and represents a major public health threat in the coming years [1]. The increase in drug-resistance and multi-drug resistant strains is a major concern since bacteria are becoming resistant to a wide range of antibiotics via natural processes and exposure following anthropogenic activities. Drug resistant bacteria acquire antibiotic resistance through mutation, horizontal gene transfer, natural transformation, transduction or conjugation. The genetic plasticity of the resistome allows antibiotic resistance genes to move rapidly through different environmental bacterial populations, communities and niches [2].

In aquaculture, antibiotics are used as growth promoters, and to prevent and treat bacterial infections in fish. However, fish do not metabolize these antibiotics efficiently and release them into the environment. It has been estimated that 75% of antibiotics administered to fish are excreted into the water [3]. Moreover, the practice of dumping animal manure directly into fish ponds as fertilizer represents a potential source of antibiotic residues and resistant bacteria in the pond environment. Antibiotics used for the treatment of human infections are also used in animal husbandry, either for therapeutic or prophylactic purposes, or to promote growth, and this contributes to the development of antibiotic resistance [4]. The health risk associated with the use of antimicrobials in aquaculture is the development and spread of antibiotic resistant bacteria and resistance genes, as well as the presence of antibiotic residues in aquaculture products [5]. The aim of this study is to assess the presence of antibiotic resistant bacteria in some fishponds used for aquaculture in the city of Yaounde.

## II. MATERIAL AND METHODS

### Study design and samples collection

The study was conducted in the city of Yaoundé, the capital of Cameroon. The practice of urban agriculture is well spread in the city of Yaoundé. aquaculture is characterized by extensive and semi-intensive systems. In these systems, fish are sometimes fed with agrifood by-products and non-conventional feeds such as kitchen waste, and animal manure such as chicken droppings and pig feces [4]. Water samples were collected in fishponds located in five districts of the city of Yaounde (Nkolbisson, Odza, Afan'ayoa, Toutouli, Etokoss). Three samplings were conducted during the months of November 2019, February and May 2020. The samples were collected using sterile plastic bottles with caps, approximately 15-25 cm below the water surface at 3 locations within the fishpond, namely at the inlet, center and outlet [6]. The collected samples were placed in an ice box at a temperature of about 4°C and transported to the laboratory for analyses. Microbial analyses were performed within 24h.

### Enumeration and identification of antibiotic resistant bacteria

Water samples (1 mL each) were suspended in 9 mL of saline solution (0.85%, NaCl). After dilution, the seeded dilution was  $10^{-3}$ ; 50 µL of each suspension was spread on Mueller Hinton (MH) agar containing one of the following antibiotics at the following concentrations: 32 µgmL<sup>-1</sup> Amoxicillin (AML, Sigma), 16 µgmL<sup>-1</sup> Tetracycline (TET, Sigma), 4 µgmL<sup>-1</sup> Ciprofloxacin (CIP, Sigma), 64 µgmL<sup>-1</sup> (8:1) Sulfamethoxazole/Trimethoprim (Sul/Trm, Sigma), 5 µgmL<sup>-1</sup> Cefixime, and 4 µgmL<sup>-1</sup> Kanamycin (KAN, Sigma) (Novo & Manaia, 2013). Petri dishes without addition of antibiotics were used to enumerate the total number of bacteria. The experiment was conducted in triplicate. All Petri dishes were incubated at 37°C for 48h. The percentage of resistance was determined based on the ratio of the total number of bacteria grown on culture with antibiotics, divided by the total number of bacteria grown on culture without antibiotics [7].

Identification of antibiotic resistant bacteria was done according to the protocol of [8,9]. It consisted of macroscopic and microscopic observation of grown colonies, cultures on specific media, and biochemical tests. After Gram staining, Gram+ bacteria were cultured on Chapman and Bile Esculin Sodium Azide (BEA) media. Gram- bacteria were grown on Methylene Blue Eosin (EMB), Cetrimide and *Salmonella/Shigella* (SS) media. Petri dishes were incubated at 37°C for 24h. Purified bacterial isolates were characterized and identified using biochemical tests as described by [8,9].

### Data Analysis

The different values obtained were reported as the mean of three replicates. The data were subjected to a one-factor analysis of variance (ANOVA) using SPSS Version 16.0 software. To determine significant differences in resistance rates, Duncan's test was used at the threshold of 0.05.

## III. RESULTS AND DISCUSSION

Resistance rates in the fishponds during the sampling months are reported in Table 1. The resistance rates ranged were 65.9% in November, 68.6% in February, and 63.1% in May, respectively for the antibiotics tested. The means were not statistically different.

**Table 1: Resistance rates of bacteria isolated from fishponds during the different months.**

Month	Resistance rate (%)
November	65,9(±3,5) <sup>a</sup>
February	68,6(±3,9) <sup>a</sup>
May	73,8 (±2,2) <sup>a</sup>

Means followed by different letters indicate significant differences in resistance rates,  $P < 0.05$ ,  $n=3$  (Duncan test).

The resistance rates ranged from 67.5% to Nkolbisson having of antibiotic resistant

Sites	Resistance rates (%)
Nkolbisson	75,7 (± 1,8) <sup>b</sup>
Odza	67,5 (±10,6) <sup>ab</sup>
Afan'ayoa	67,9 (± 10,9) <sup>ab</sup>

in the sampled sites 75.7%, the site of the higher proportion bacteria.

**Table 2: Resistance rates of bacteria isolated in the different sampling sites.**

<b>Toutouli</b>	68,1 (± 9,8) <sup>ab</sup>
<b>Etokoss</b>	67,9 (± 2,1) <sup>a</sup>

Means followed by different letters indicate significant differences in resistance rates,  $P < 0.05$ ,  $n = 3$  (Duncan test).

The greater presence of drug resistance bacteria during the month of May which is the rainy season is in accordance with the studies of [10] in Bangladesh on the assessment of antibiotic-resistant bacteria in pond water associated with integrated poultry aquaculture. This could be explained by the fact that runoffs follow rains and there is an influx of contaminants in the fishponds, including antibiotic resistant bacteria. It has been reported that contamination from soil, anthropogenic sources and aquatic environments occurs mainly during the rainy season, which accelerates mixing and horizontal gene transfer in microbial ecosystems, favoring the spread of resistant bacteria [11].

The Nkolbisson site (75.7%), is located downstream houses, and during rains, the water that runs off from everywhere is discharged into the site, and this increases its pollution level compared to the other sites that have a flat relief. A greater prevalence of antibiotic resistant bacteria was expected. For a better management of the fishponds, farmers should install water filtration systems to minimize pollution from surrounding water sources.

The rate of resistance of the different isolates to the tested antibiotics is reported in Table 3. Resistance rate to amoxicillin was the highest (86%), followed by cefixime (81%), tetracycline (75%), sulfamethoxazole / trimethoprim (65%), kanamycin (62%) and ciprofloxacin (48%).

**Table 3: Resistance rates of the bacteria isolates to the antibiotics tested**

Antibiotics	Resistance rate (%)
<b>Amoxicillin</b>	86,1(±7,2) <sup>c</sup>
<b>Ciprofloxacin</b>	48,2(±2,7) <sup>a</sup>
<b>Tetracycline</b>	75,2 (± 7,5) <sup>bc</sup>
<b>Sulfamethoxazole /Trimethoprim</b>	64,5 (± 1,7) <sup>b</sup>
<b>Cefotaxime</b>	80,9 (± 8,9) <sup>c</sup>
<b>Kanamycine</b>	61,9 (± 1) <sup>b</sup>

Means followed by different letters indicate significant differences in resistance rates,  $P < 0.05$ ,  $n = 3$  (Duncan test).

Amoxicillin was the least effective antibiotic with a resistance rate of 86.1%. This result is in accordance with those of [12] who worked on the assessment of bacterial resistance to antibiotics in integrated aquaculture environments in Ghana and obtained a resistance rate of 100% to ampicillin and penicillin. Less susceptibility of bacteria to amoxicillin is due to the fact that these molecules have been used for a long time as the first line antibiotics against all kinds of infections in human and veterinary medicine. Moreover, fecal matter is a major contaminant of aquatic environments and is considered to be a major carrier of  $\beta$ -lactam resistance genes for enterobacteria in aquaculture [13]. Ciprofloxacin is an antibiotic belonging to the quinolone family. Low rates of resistance to ciprofloxacin (48.2%) have been observed. This could be explained by the fact that ciprofloxacin is a second generation extended spectrum antibiotic that remains effective. Moreover, the only mechanisms of resistance to these antibiotics described are essentially chromosomal with a very low probability of occurrence (occurs on average every  $10^6$ - $10^9$  divisions). Although recently described mechanism such as active efflux is emerging, they confer low levels of resistance to this family. [5] conducted a study on the antibiotic susceptibility profile of bacteria isolated from fish ponds in the Niger Delta region of Nigeria, the susceptibility of bacterial isolates to ciprofloxacin was 50% for earthen ponds. This shows the effectiveness of this antibiotic. It is therefore essential to insist on the rational use of quinolones and fluoroquinolones to decrease the selection pressure of acquisition of genes coding for resistance against these antibiotics as there is a risk of dissemination of resistance to this therapeutic family.

Fifty-six antibiotic resistant bacteria belonging to the following species were identified, *Pseudomonas* spp., *Staphylococcus aureus*, *Shigella* spp., *Enterococcus* spp., *Enterobacter* spp., *Escherichia coli*, *Streptococcus* spp., *Salmonella* spp., *Bacillus cereus*, *Micrococcus* spp.

The frequency of resistance of the bacterial isolates to all antibiotics tested is reported in Table 4. *Staphylococcus aureus* presented the greater rate of resistance (32.1%), followed by *Shigella* spp. (23.2%), *Escherichia coli* (18%), *Enterococcus* spp. (10.7%), *Streptococcus* spp. and *Salmonella* spp. (8.9%), *Pseudomonas* spp. (7.1%), *Bacillus cereus* (3.6%), *Micrococcus* spp., and *Enterobacter* spp.

**Table 4: Frequency of resistance of antibiotic resistant bacteria isolated.**

Antibiotic resistant bacteria	Frequency of resistance (%)
<i>Staphylococcus spp.</i>	32,1%
<i>Pseudomonas spp.</i>	7,1 %
<i>Streptococcus spp.</i>	8,9 %
<i>Shigella spp.</i>	23,2 %
<i>Salmonella spp.</i>	8,9 %
<i>Enterococcus spp.</i>	10,7 %
<i>Bacillus cereus</i>	3,6 %
<i>Micrococcus spp.</i>	1,8 %
<i>Enterobacter spp.</i>	1,8 %
<i>Escherichia coli</i>	18 %

Five bacterial genera out of the ten-isolated belonged to the *Enterobacteriaceae* family, namely *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Enterococcus spp.*, and *Enterobacter spp.* The presence of these bacteria normally found in the digestive tract of warm-blooded animals in the fishponds is consequent to fecal contamination having human or animal origin [14], their presence would be consistent with the use of organic manure, especially poultry. The majority of the aquaculture farmers interviewed use poultry manure to fertilize the ponds. This manure is dumped into the ponds for fertilization, which favors the phenomenon of antibiotic resistance. Antibiotics belonging to aminosides, sulfonamides, quinolones, macrolides, tetracyclines and penicillins are used in poultry in Cameroon [15]. These antibiotics are found in chicken droppings, which can promote the spread of antibiotic resistance. The use of chicken manure to fertilize fishponds could explain their high prevalence [5]. Fish infected with these bacteria, can be a vehicle for disease transmission to humans, the risk is further increased if these bacteria are resistant to antibiotics. The permanent exposure of fish to manures used as organic fertilizers in aquaculture could lead to risks of contamination of fish by antibiotic resistant bacteria, which is a threat to public health because they can also be transferred to humans. The presence of antibiotic resistant bacteria *Pseudomonas spp.* *Staphylococcus spp.* *Salmonella spp.*, responsible for gastroenteritis, diarrhea and typhoid is alarming since they can infect fish and humans [16]. To reduce or limit antibiotic resistant bacteria input into fishponds, the farmers could dry chicken manure. Some studies have demonstrated that pretreatment of animal excreta by sun drying before use, reduce the prevalence and bacterial loads in samples associated with the use of animal fertilizers in a dry state, compared to fertilizers (manure and dung) not subjected to drying [17].

Multidrug-resistant bacteria have been isolated from fishponds. Their presence in waters represents a serious public health concern as they can cause treatment failures in case of outbreaks in fish and humans. These multidrug-resistant bacteria pose a real danger to populations that come into contact with these waters [18]. Fish typically succumb to opportunistic bacterial infection due to physiological imbalance and stressors such as nutritional deficiency, poor water quality, and overstocking are predisposing factors for the development of fish infections. The number of antibiotic resistant bacteria identified in the fishponds could be insignificant given the few tests conducted. Systematic monitoring of antimicrobial resistance using advanced techniques such as metagenomics and whole genome sequencing could provide a better information regarding diversity, prevalence and evolution of multidrug resistant bacteria in fishponds.

### III. CONCLUSION

The results obtained from this study showed that urban aquaculture might represents a strong vector for antibiotic resistant bacteria dissemination, the majority of drug resistant bacteria isolated from fishponds belong to the *Enterobacteriaceae* family which is a public health concern, regarding poor water and sanitation in the city. Further studies are needed to evaluate the extend of the problem. There is an urgent need to regulate aquaculture practices to protect farmers and consumers.

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