

*Full Length Research Paper*

## **Occurrence of multidrug-resistant bacteria in aquaculture farms in Côte d'Ivoire (West Africa)**

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Received 28 December, 2019; Accepted 23 April, 2020

**Aquaculture provides a significant proportion of the fish consumed around the world. In West Africa, aquaculture is an important economic sector. However, several diseases with high fish mortality are caused by bacterial infections. Due to the lack of surveillance in aquaculture, this study investigated the presence of bacteria in fish farms. The purpose of the study was to isolate bacteria in aquaculture (Ivory Coast). Two hundred and forty fishes and water samples were collected from the pond of two fish farms. Fish was scraped, then, dissected to collect their gills and intestines. Bacterial culture was done for the detection of many species. Isolate identification was done using biochemical tests (API20E) and MALDI-TOF tests. Also, 1696 bacteria strains were isolated, 70.9% of strains were from the fish organs and 29,1% from the water samples. The higher colonization rate was observed in water and on fish's surface. No statistical difference was observed between the two farms. Seven major species were isolated in both farms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter hormaechei*, *Enterococcus faecalis*, *Citrobacter freundii*, *Morganella morganii* and *Bacillus cereus*. The major isolated strains were *Enterobacter hormaechei*, *Enterococcus faecalis* and *Escherichia coli*. Multi-resistance for 3 classes of antibiotics was observed in some of those strains. This investigation shows microbiological risks for aquatic animals and humans who are in interaction with fish farms.**

**Key words:** Aquaculture, multidrug-resistant strains, fish, West Africa.

### **INTRODUCTION**

Aquaculture belongs to the food industry with an average growth rate of 6.2% in the past years (FAO, 2016). It is an activity that contributes to the production of foods of high nutritional value, generating employment and economic income for the world population (Gabriel et al.,

2007). In addition, it strengthens the source of inputs for the food industry and foreign exchange for the country (Kouadio et al., 2019). In Africa, particularly in Côte d'Ivoire, aquaculture has an important place in the food industry and for consumers (Koumi et al., 2017;

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**Figure 1.** *Oreochromis niloticus* organs collected in two aquaculture farms of Dabou, West Africa, Côte d'Ivoire; **a:** Whole fish (*Oreochromis niloticus*); **b:** Swabs on the surface of fish; **c:** Gills; **d:** Intestine.

Weichselbaum et al., 2013; Yao et al., 2017). In all aquaculture operations in the country, Tilapia is the major specie reared with frequencies depending on the system. One of the biggest threats to the aquaculture sector is infectious diseases (Abowei and Briyai, 2011). Antibiotics are used in aquatic ecosystems to control the bacteria responsible for infectious diseases (Watts et al., 2017; Ouattara et al., 2014; Salah and Aqel, 2014). Excessive use of antibiotics has led to the resistance of pathogenic bacteria. (Gao et al., 2012; Miller and Harbottle, 2017). Vibriosis, Photobacteriosis and Furunculosis are among other major diseases of marine and estuarine fish in natural and in aquaculture (Toranzo et al., 2005). The agents of Vibriosis are several species of *V. anguillarum*, *V. vulnificus*, *V. alginolyticus* and *V. salmonicida*. Photobacteriosis is caused by Photobacterium (Mookerjee et al., 2015). The surveillance of fish disease is under estimated in West Africa and the risk of dissemination is very high, as countries with limited resources do not have veterinary clinics providing clinical and biological diagnostics to monitor the use of antibiotics in animals (Ouedraogo et al., 2017). In West Africa, antibiotic resistance induces the emergence of resistant anterobacteria (Pitout and Laupland, 2008). Several studies have shown that 90% of bacterial strains in marine environment are resistant to more than one antibiotic and 20% are resistant to at least five antibiotics (Kouadio et al., 2017; Benie et al., 2017; Dib et al., 2018). Previous studies have demonstrated the extent of the circulation of bacterial multi resistance. It has been shown

in certain animals such as chickens (Koga et al., 2015) in the natural environment and in artificial water systems. (Ouattara et al., 2014). In Côte d'Ivoire, few data are provided on the detection of bacterial multi resistance in aquaculture environments. Previous studies carried out in the DABOU area have focused more on pathogenic bacteria and less on those with antibiotic resistance (Kouadio-Ngbesso et al., 2019). However, surveillance of bacterial multi resistance has become a major concern throughout the world. this study investigated the presence of multi-drug resistant bacteria in two fish farms in Dabou, in the south of Abidjan.

## MATERIALS AND METHODS

### Study area

Water and fish (*Oreochromis niloticus*) samples were collected from two fish farms in Dabou, located in the South of Ivory Coast (fish farm 1 with GPS data: 5° 19' 34,44" N, 4° 22' 0.45" W; fish farm 2: 5°18'41.51" N, 4° 24'51.66" W). The study area is characterized by different aquatic ecosystem (lagoon and river) and with natural vegetation. Around these aquaculture zones, some human activities exist. Each fish farm area covers 1 hectare with 12 rectangular ponds (Figure 1).

### Sampling in the aquaculture farm

Sampling was done during six months from November 2017 to April 2018 with five ponds per fish farm. Samples were collected once a month. During each visit four water and fish samples were collected

**Table 1.** Distribution of collected samples.

Type of sample	Farm 1	Farm 2	Total
	Number of samples (n)	Number of samples (n)	
Water	120	120	240
Fishes organs (swabs, gills and intestine)	120	120	240
<b>Total (%)</b>	<b>240 (50%)</b>	<b>240 (50%)</b>	<b>480 (100%)</b>

from different points in each of the five ponds of the fish farms. Water samples were taken using sterile glass vials attached to a rope and fish samples were taken using mowing nets. Afterwards, 100 ml of water sample was taken from four different points in each pond. At each sampling period, Fish were sampled by rubbing its surface with swab. Next, the fish were dissected to remove their gills and intestines according to Adingra et al. (2010). A total of 240 were collected (Table 1). The water and fish samples were transported to the laboratory in a cooler containing (4° C) cold accumulator.

## Bacteriological examination

### Isolation and biochemical identification

Fishes were skinned and gills and intestines were removed. A small portion of the organs and the swabs were transferred into sterile 3 ml of PBS1X (pH= 7.2). The solution was mixed and 100 µl were inoculated into 3 ml of Luria Bertani broth and incubated at 37°C for 24 h. After 24 h cultures were inoculated on different media: trypticase soy agar (TSA) for Enterobacteriaceae, Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) for *Vibrio* isolation, Eosin Methyl Blue Agar (EMB) for Gram negative bacteria and selective media for *Pseudomonas* group Cetrimide Agar (biolab, cab. 20500, lot: cab 090419072). After 18-24 h of incubation at 37°C, bacteria colonies were selected to perform Gram staining (Saleh et al., 2017). Bacterial identification was done by API 20E kits (BioMerieux, France).

### MALDITOF analysis

Bacteria strains were identified by MALDI-TOF (Biomerieux, VITEK MS) according to the principle described by Carbone and Nassif (2011). The bacterial strains to be analyzed were cultured on microbiological agar and incubated at 37°C for 24 h. The colonies obtained were used for analysis with MALDITOF. The method is based on the ionization of bacterial proteins by a laser beam and the creation of characteristic peaks (spectrum). The preparations were made in "sandwich" form: the sample is deposited on a matrix film before being covered by a final matrix layer. The plate carrying the samples is placed in the spectrometer where they are subjected to the laser beam. From a database of spectra, the software searches for the corresponding species of bacteria according to a reliability index between two spectra. The confirmation of the strains is data based (VITEK MS 3.1).

### Antibiotic susceptibility of strains

Fresh bacterial colonies were added to 2 ml of physiological saline. The density of the bacterial suspension was adjusted to 0.5 Mc Farland it was inoculated onto the surface of Mueller Hinton agar (OXOID, CM: 0337). Selected antibiotic discs (Table 3) were placed

on the agar and incubated at 37°C for 24 h. The diameter interpretation was based on the EUCAST 2019 (European Committee on Antimicrobial Susceptibility Testing V.1.0) recommendations. The sensitivity or resistance of bacteria to antibiotics is evaluated by comparing the inhibition diameters to the minimum inhibition concentration of the discs. If the measured inhibition diameter on the agar is greater than the minimum inhibition concentration of the disc, the bacterium is sensitive to the antibiotic; otherwise it is resistant (Guessens et al., 2013).

## RESULTS

### Bacterial dissemination in fishes and in water samples

Seven bacterial species were isolated from water and in fish organs collected from two aquaculture farms. *Enterobacter hormaechei* (*hor*), *Enterococcus faecalis* (*fac*), *Citrobacter freundii* (*cit*), *Bacillus cereus* (*bac*), *Pseudomonas aeruginosa* (*psd*), *Escherichia coli* (*col*) and *Morganella morganii* (*mor*) were isolated and confirmed by biochemical tests and by MALDI-TOF analysis. The strains showed resistance to antibiotics. These isolated strains represent potential sources of pathogenicity for humans. Swab and water samples have similar colonization rates with 29.7 (504/1696) and 29.12% (494/1696), respectively; whereas intestine and gills showed a lower colonization rate by 15% (Table 2). Bacterial species in the aquaculture farms were: *Enterobacter hormachaei*, *E.coli*, *B. cereus*, *Enterococcus faecalis* and *M. morganii* with presence of 16, 15.9, 14.9, 14.2, and 14% of the samples, respectively (Table 2). The ANOVA 1 test showed no significant difference between the two farms for the presence of bacterial strains.

### Antibiotic susceptibility testing

Isolated bacterial strains were tested for sensitivity to different families of antibiotics (Table 3). The bacteria *E. coli*, *Citrobacter freundii*, *M. morganii*, *E. faecalis* and *E. hormachei* are subjected to the same antibiotics. On the other hand, strains of *Pseudomonas aeruginosa* were tested with another group of antibiotics. The results showed that 44.9% (31/69) of the strains were resistant to tested antibiotics and 55.9% were susceptible to those

**Table 2.** Distribution of bacterial strains isolated in farms according to the different types of samples.

Sample type	Site	Number of isolated bacteria strains (n)							Total
		<i>E. coli</i>	<i>C. freundii</i>	<i>P. aeruginosa</i>	<i>M. morgani</i>	<i>B.cereus</i>	<i>E.faecalis</i>	<i>E. hormaechei</i>	
Swab	F1	45	33	20	37	37	30	45	247
	F2	39	41	33	38	39	26	41	257
Gill	F1	22	17	14	23	26	31	36	169
	F2	27	20	16	17	25	35	28	168
Intestine	F1	23	24	20	31	32	32	29	191
	F2	32	22	20	23	19	32	22	170
<b>Total 1</b>		<b>188</b>	<b>157</b>	<b>123</b>	<b>169</b>	<b>178</b>	<b>186</b>	<b>201</b>	<b>1202</b>
Water	F1	43	32	25	35	35	30	41	241
	F2	39	40	30	35	40	25	44	253
<b>Total 2</b>		<b>82</b>	<b>72</b>	<b>55</b>	<b>70</b>	<b>75</b>	<b>55</b>	<b>85</b>	<b>494</b>
<b>Total 3</b>		<b>270</b>	<b>229</b>	<b>178</b>	<b>239</b>	<b>253</b>	<b>241</b>	<b>286</b>	<b>1696</b>
<b>(%)</b>		<b>15,9%</b>	<b>13,5%</b>	<b>10,4%</b>	<b>14%</b>	<b>14,9%</b>	<b>14,2%</b>	<b>16,8%</b>	<b>(100%)</b>

F1: Farm 1; F2: Farm 2.

**Table 3.** Distribution of antibiotics susceptibility of isolated bacteria strains.

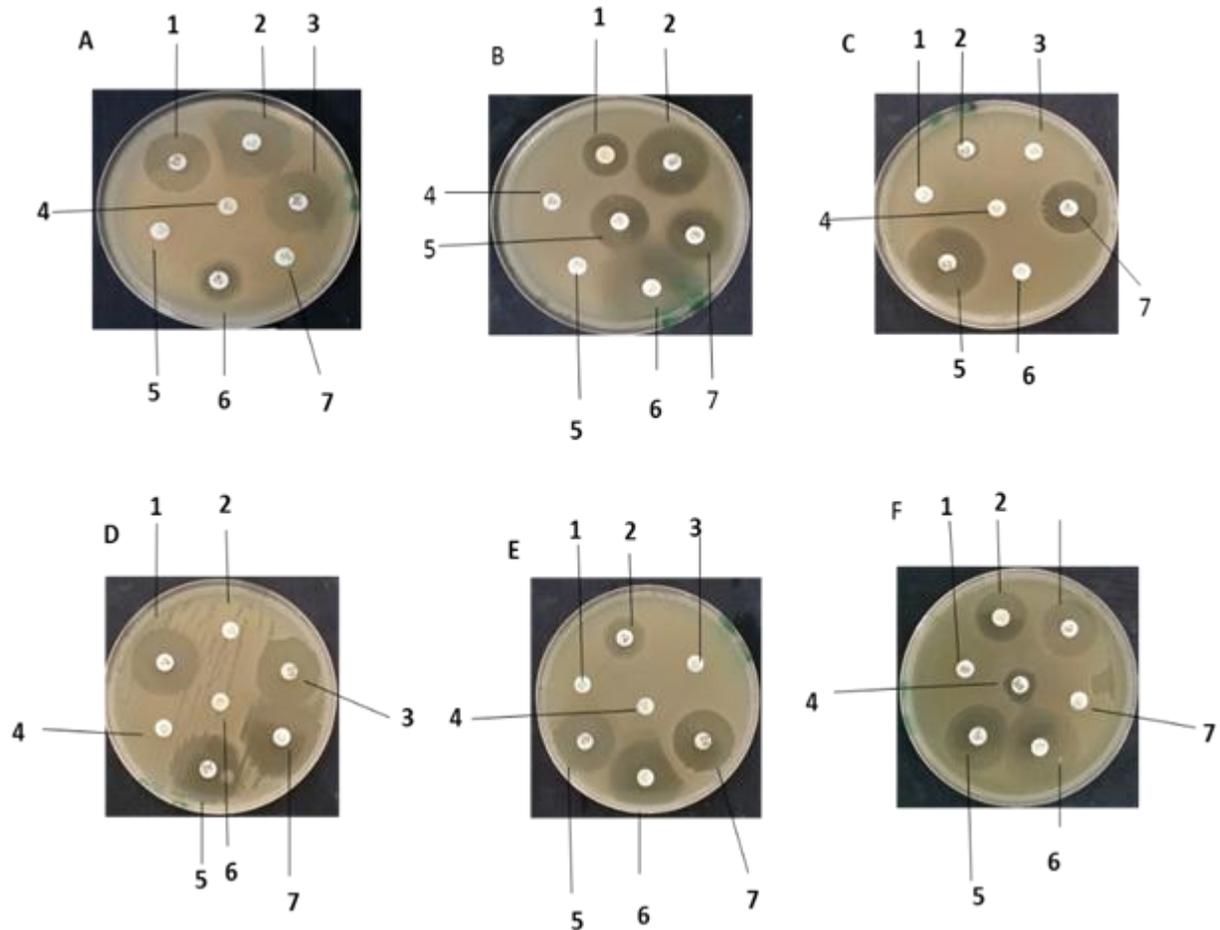
Family	Antibiotics	Number of tested strains (n)	Number of resistant strains (R)	Number of sensitive strains (S)
Penicillins	Amoxicilline/Acid clavulanic (AMC)	4	4	0
	Ticarcilline/Acid clavulanic (TCC)	1	1	0
Penicillins/Monobactams	Piperacilline/Tazobactam (TZP)	5	5	0
	Piperacillin (PIL)	5	5	0
Penicillins	Ticarcillin (TIC)	5	5	0
	Ceftazidim (CAZ)	5	1	4
Cephalosporins	Cefoxitin (FOX)	5	3	2
	Aztreonam (ATM)	5	1	4
Monobactams	Imipenem (IPM)	6	0	6
Fluoroquinolons	Ciprofloxacin (CIP)	5	0	5
	Acid nalidixic (NAL)	3	1	2
Aminoglycosides	Gentamicin (GMI)	5	0	5
Tetracyclines	Tigecyclin (TCG)	5	1	4
Aminoglycosides	Fosfomycin (FSF)	5	1	4
Sulfonamidess	Cotrimoxazole (SXT)	5	3	2
	<b>Total</b>	<b>69</b>	<b>31</b>	<b>38</b>
			<b>(44.92%)</b>	<b>(55.07%)</b>

antibiotics (Table 3). *E. coli*, *M. morgani* and *P. aeruginosa* strains showed resistance against 3 different antibiotics families (Figure 2A, D, E); while *E. hormachei* and *E. faecalis* for 2 antibiotics (Figure 2B and F) and *C. freundii* strains have multiple resistance against five antibiotics (Figure 2C). Multi-drug resistance for 3 classes

of antibiotics is observed in some strains (Figure 2A to F).

## DISCUSSION

The bacteriological evaluation of fish and water samples



**Figure 2.** Antibiotic susceptibility for isolated strains in aquaculture farms. **A:** *E. coli* (1:IPM; 2: ATM;3: CAZ;4: AMC;5: PIL;6: FSF;7:TZP); **B:** *E. hormaechei* (1: TGC; 2: SXT; 3: FOX;4: NAL;5: TIC;6: CIP;7: GMI); **C:** *C. freundii* (1: ATM; 2: CAZ; 3: TZP; 4: AMC; 5:IPM; 6:PIL ;7: FSF); **D:** *M. morganii* (1: FSF; 2: PIL;3: IPM; 4: TZP; 5: CAZ; 6: AMC; 7: ATM); **E:** *P. aeruginosa* (1: TZP; 2: FSF; 3: PIL; 4: TCC; 5: CAZ; 6: AMC; 7:IPM); **F:** *E. faecalis* (1: SXT; 2:TGC; 3:GMI; 4: NAL; 5: FOX; 6: CIP; 7:TIC).

collected from two farms revealed different species of bacteria. The bacterial colonization rate showed that *E. coli* was the most isolated bacteria followed by *P. aeruginosa* and *E. faecalis*. The presence of these bacteria has been shown in other studies that also reported the presence of *Bacillus cereus* with multi-resistance in aquaculture tanks (Gao et al., 2012).

This high prevalence of these three bacteria could be linked to contamination of the feeding sources of fish ponds. Several human activities (farming, laundry, fecal matter, market gardening) around aquaculture areas contaminate natural waters such as rivers and lagoons. These waters, which are prone to microbial contamination, are used as a source of water for ponds and are sometimes untreated (Gabriel et al., 2007; Benie et al., 2017). Rasool et al. (2017) reported the presence of *B. cereus* in fishes in India, suggesting that the detection of bacteria in water and farmed fish is not

limited to a single bacterial species.

*E. faecalis*, which showed a high colonization rate in this study, preferentially this bacterium is found in the intestines of warm-blooded animals. Finding these bacteria in fish that is a cold-blooded animal (poikilotherm), could be explained by human contamination. The presence of *E. coli* in the fish indicates fecal contamination of the biotope. Saeidi et al. (2018) have reported that the presence of *E. coli* and other enteric bacteria indicate fecal contamination. *Bacillus cereus* is one of the bacteria responsible for food poisoning. The presence in aquaculture suggests the persistence of this bacterium in the environment. Different genes responsible for enterotoxin production in *B. cereus* have been characterized (Ehling-Schulz et al., 2019). So far, no study has been carried out to relate the presence of virulent genes and enterotoxin production in case of isolates of *B. cereus* from fish.

Environmental factors can influence the bacterial colonization of pond water and fish (Fister et al., 2016). The detection of pathogenic bacteria in fish farms could reflect the list of pathogens in fishes (Toranzo et al., 2005; Soto-Rodriguez et al., 2013) and their geographical distribution of diseases and particularly in sub-Saharan Africa with countries with similar fish farming practices. Some studies have reported identical results for bacterial diseases in fishes (Abowei and Briyai, 2011; Eshetu et al., 2014). The detection of multi-resistant strains in this study correlates with the findings of Ouattara et al. (2016) with the dissemination in the aquaculture environment in Cote d'Ivoire. As a consequence of abusive and uncontrolled use of antibiotics for medical, veterinarian and food production, the increase of MDR is a general trend in West Africa (Gao et al., 2012; Devarajan et al., 2015). The rate of bacterial colonization in fish farms could influence yield (Bentzon et al., 2016) as it would result in a loss through fish mortality. It would be necessary to ensure the quality of the water used in the ponds or their source. In fact, there is no boundary between the different environments (industrial, hospital agricultural, animal) and the populations (Rajani et al., 2016; Toule et al., 2017). The results confirm the presence of MDR strains in aquaculture and show the need to investigate the occurrence of enterotoxigenic *B. cereus*, *E. coli* and other bacteria and to study the relationship between their presence and the presence of diarrheal enterotoxin.

## Conclusion

The importance of aquaculture production to provide future fish demands for human consumption is evident. One of the biggest challenges is the surveillance and control of fish production. In Africa, there is lack of surveillance in the food production system. There is a need to correlate human, animal and environment health surveillances. The presence of potential pathogens which are multidrug-resistant in aquaculture farms poses a considerable threat to public hygiene. The distribution of bacteria strains in water and in fish organs correlated in both farms. This study has demonstrated the presence of resistant strains in aquaculture farms and suggests the transmission of bacteria from nearby environments to the fish farms.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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