Microbial Assessment and Antibiotic Susceptibility Profile of Bacterial Fish Isolates in an Aquaculture Production Site in Mefou Afamba Division of Cameroon

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Abstract: The practice of integrated fish farming and the use of local fish meal and manure for fish feeding in Cameroon constitute potential sources of resistant pathogenic bacteria in the fish pond environment. Therefore, a periodical and constant monitoring of the microbiological quality of fish pond is imperative. This study was to assess the microbial contamination of Mfou aquaculture production site and evaluate the antibiotic resistance profile of bacterial fish isolates. Samples of pond water ($n = 36$), sediment ($n = 36$), fishmeal ($n = 12$) and African catfish (*Clarias gariepinus*), kanga (*Heterotis niloticus*) and Nile Tilapia (*Oreochromis niloticus*) ($n = 36$, each) were collected to determine TVAC (Total Viable Aerobic Bacterial Count), FC (Fungal Count), SAC (*Staphylococcus aureus* count), TCC (Total Coliform Count) and FCC (Feacal Coliform Count). The fish skin isolates of *S. aureus*, *Enterobacter sakazakii*, *Citrobacter freundii*, *Serratia fonticola*, *Klebsiella oxytoca*, *Proteus* spp., *Aeromonas hydrophila*, *Kluyvera* spp., *Moraxella* spp., *Pasteurella multocida* and *Pseudomonas fluorescens* were tested against penicillin G (10 µg), chloramphenicol (30 µg), sulfamethoxazole/trimethoprim (25 µg), erythromycin (15 µg), tetracycline (30 µg), using the disk diffusion method. Results reveal a heavy contamination of fish farms with microbial load above the recommended limits. Our study indicates that fish ponds are sources of zoonotic pathogens underlining their epidemiological and clinical relevance. All bacterial isolates were multiresistant with a multiple antibiotic resistance index above 0.2. These data raise concern about the microbial contamination of aquaculture and associated public health risks in Cameroon.

Key words: Microbial contamination, antibiotic susceptibility profile, aquaculture, pond fish, zoonotic pathogens, Cameroon.

1. Introduction

Despite a high rate of fish importation, consumers' demand for fresh fish is on the increase in Cameroon [1]. To address this gap, the Cameroon government has promoted intensive fish farming both for local supply and for boosting the income of fish farmers [1]. Majority of farmers practiced earthen fish pond using integrated fish farming system with poultry, piggery and crop farming [2]. Fish feeding practices consist in the use of locally formulated feeds and animal manure. Unfortunately, integrating fish farming with poultry, goat, and crop farming (like at the Mfou aquaculture production site) and using local manufactured fish meal and animal manure represent sources of microbial contamination of the fish pond environment. The release of high concentration of opportunistic and pathogenic bacteria into the fish ponds may eventually affect the quality of pond water and sediment which in

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return can compromise the health and safety of fish therein [3]. Therefore, microbial contamination of fish pond poses a threat not only to fish and human health but also to the environment [3, 4]. Moreover, the non-referral of more than 75% of fish farmers in Cameroon to a veterinarian for prescription [2] may promote drug resistance among microbiota of fish pond [4]. Additionally, the location of poultry farms near fish ponds in Cameroon may also encourage antimicrobial resistance in the aquaculture environment given the high use of antibiotics in poultry [5, 6]. In this regard, the presence of antibiotic residues reported in poultry products such as chicken litter manure can drive the development of multidrug resistant bacteria in fish pond during spillage [6].

Given the fact that water-borne or fish-borne diseases such as diarrhea and cholera are reported every year in Cameroon, fish farming requires periodical and constant monitoring to avoid contamination of the aquaculture environment with resistant pathogenic organisms. The present study was aimed to assess the microbiological quality of fresh fish, pond water, pond sediment and fish meal at the Mfou aquaculture production site.

2. Materials and Methods

2.1 Study Area

The study was conducted between June and November 2020 in an intensive fish production farm at Mfou, the divisional headquarter of the Mefou Afamba division, located 60 km away from Yaounde, the capital city of Cameroon. Fish ponds in this study were all owned by private farmers. The farmers practiced integrated fish farming system with poultry, goat, and crop farming. Farmers equally drain the pond water into the environment every fish harvest session.

2.2 Sample Collection

Samples of African catfish (*Clarias gariepinus*), kanga (*Heterotis niloticus*) and Nile Tilapia (*Oreochromis niloticus*) (*n* = 36, each) were collected

(at different points) from three different fish ponds in clean and sterile iceboxes containing water from the respective ponds. Pond water samples (10-15 cm below the water surface) (*n* = 36) from three different earthen ponds connected to different water sources (stream water, swamp water, stream and swamp water) were collected aseptically in duplicates in pre-sterilized bottles (250 mL); sediment-mud samples (*n* = 36) were collected from the bottom (at the different points) of the ponds in sterilized screw-capped glass bottles (500 mL). Fish meal (*n* = 12) was also collected from the farmers during the three field trips. All samples were collected in duplicates. With the exception of the fish species (transported in their natural environment), other samples including water, mud and fishmeal were transported immediately to the microbiology unit of the Research Centre for Food and Nutrition in ice-packed containers for microbiological analysis.

2.3 Sample Processing

Upon arrival at the laboratory, fish were classified according to species and fish ponds and disinfected by dipping in 70% alcohol for 2 min, then rinsed three times with sterilized distilled water [7]. The gills, the intestines and the skin were separated from the fish and ground separately in a sterile stomacher bag. The pond sediment (mud) collected from different points of each pond and fish meal in different bags were also mixed separately using sterile stomacher bag. The water samples collected at different points of each fish pond were mixed in a sterilized conical flask (1,000 mL).

2.3.1 Serial Dilution of Samples and Inoculation of Media

Under sterile condition of laminar flow, 25 g of each solid sample (skin, intestine, gills, pond sediment and fish meal) and 25 mL of pond water were introduced into 225 mL of cooled sterile peptone water and homogenized using a vortex. The solution was allowed to stand at room temperature for 15-30 min for revivification purposes. Using a 1,000 μL

micropipette, 1 mL of each sample solution was introduced into 9 mL sterile distilled water to make 10^{-1} dilution. From 10^{-1} dilution, 1 mL of the mixture was transferred to the second tube to make 10^{-2} dilution. These procedures were taken for the successive dilution in a similar way to give up to 10^{-10} dilution. The diluent was inoculated on culture plates of PCA (Plate Count Agar), SA (Sabouraud Agar), MSA (Mannitol Salt Agar), MCA (Maconkey Agar), EMB (Eosin Methylene Blue) agar, using pour plate method for TVAC (Total Viable Aerobic Bacterial Count), FC (Fungal Count), SAC (*Staphylococcus aureus* Count), TCC (Total Coliform Count) and FCC (Faecal Coliform Count), respectively. For TCC and FCC, the diluent was first inoculated in brilliant lactose green broth and then introduced into MCA and EMB upon incubation at 37 ℃ and 44.5 ℃ for 24 h, respectively. The inoculated SA was incubated at 30 ℃ for 3-5 days.

2.4 Isolation and Identification of Bacteria from the Fish Skin

The isolation and identification of bacteria were limited to the edible parts (fish skin), considering that the gills and intestine are not consumed by the Cameroonian population. After incubation, the colonies were subcultured on fresh nutrient agar plates to obtain pure cultures for identification purposes. Colonies were presumptively identified using standard techniques based on their morphology and colonial and Gram staining characteristics. The presumptive colonies of *S. aureus* were confirmed using oxidase and catalase tests whereas enterobacteria were confirmed using API-20 E kit (BioMérieux, France). The instructions given by the manufacturer (BioMérieux, France) were strictly followed. The results were read after incubation at 37 ℃ for 18-24 h and interpreted using Apident software.

2.5 Antibiotic Susceptibility Testing

Bacterial isolates from the fish skin were assessed for their susceptibility to different antibiotics using the disk diffusion method according to method described by Clinical and Laboratory Standards Institutes [8] on MHA (Mueller-Hinton Agar). The panel of antibiotics (UK) used for testing was: penicillin P (10 μg), chloramphenicol C (30 μg), sulfamethoxazole-trimethoprim SXT (25 μg), erythromycin E (15 μg) and tetracycline TE (30 μg). A bacterial lawn was prepared by transferring 4-5 bacterial colonies to a glass tube containing 5 mL sterile physiological water with a sterile inoculating loop. The suspension was vortexed and visually matched with 0.5 MacFarland standards. The suspension was poured upon the surface of plate agar and then removed. The antibiotic disks with known concentration were placed upon the bacterial lawn using sterile forceps. The disks were then gently pressed down onto the agar. Care was taken to prevent overlapping of the zones of inhibition and possible error in measurement by distancing the antibiotic disks 24 mm away from each other and from the edge of the plate agar. The plates containing the bacterial lawn and the antibiotic disks were then incubated at 37 ℃ for 18-24 h. The diameter of the zones of inhibition around each antibiotic disk was measured with a ruler and recorded to the nearest millimeter and isolates were classified as susceptible, intermediate or resistant according to the zone diameter interpretative standards of the Clinical and Laboratory Standards [8]. MAR (Multiple Antibiotic Resistance) index was then determined for each isolate by dividing the number of antibiotics to which an isolate is resistant with the total number of antibiotics tested [9]. MAR index value > 0.2 indicates that the isolates were isolated from high-risk sources [10].

2.6 Statistical Analysis

The bacterial counts in pond water, pond sediment, fish meal and fish intestines, gills and skin were statistically analyzed by one-way analysis of variance using IBM SPSS Statistics 22. The differences were considered significant for p values < 0.05.

3. Results

3.1 Microbial Load of Samples

Results in Table 1 indicate that the microbial load of samples (fish parts, mud and pond water) was generally high. The TVAC of samples ranged from 4.70 $\log_{10}$ CFU g^{-1} to 8.49 $\log_{10}$ CFU g^{-1} whereas the total FC varied from below $\log_{10}$ CFU g^{-1} to 6.58 $\log_{10}$ CFU g^{-1}. In addition, the TCC ranged from 3.6 $\log_{10}$ CFU g^{-1} to 7.7 $\log_{10}$ CFU g^{-1} while FCC ranged from 3.0 $\log_{10}$ CFU g^{-1} to 7.4 $\log_{10}$ CFU g^{-1}. Generally, the SAC, which varied from 3.38 $\log_{10}$ CFU g^{-1} to 7.14 $\log_{10}$ CFU g^{-1} was slightly lower than the contamination rate of samples with other group of indicator organisms. Results also reveal that there was no statistically significant difference ($p > 0.05$) between microbial load of fish irrespective of the fish species and the pond type. However, total FC and FCC significantly ($p < 0.05$) varied between fish parts (intestine gills and skin). The microbial load of fish meal ranged from below $\log_{10}$ CFU g^{-1} to 7.06 $\log_{10}$ CFU g^{-1} (data not shown).

The mean total viable bacterial count of fish irrespective of the parts and species (Table 2) was beyond the permissible level (5.00 $\log_{10}$ CFU g^{-1}). While the mean FC of water was 3.24 $\log_{10}$ CFU mL^{-1} that of fish ranged from 2.72 to 3.24 $\log_{10}$ CFU g^{-1}. On the other hand, the mean TCC for pond water (5.13 $\log_{10}$ CFU mL^{-1}) and for fish (4.47 to 5.06 $\log_{10}$ CFU g^{-1}) was far beyond 2-3.7 $\log_{10}$ CFU mL^{-1} and 2 $\log_{10}$ CFU g^{-1}, the recommended limits for freshwater and fresh fish respectively. The mean FCC of fish skin (3.7-3.9 $\log_{10}$ CFU g^{-1}) and that of pond water (3.76 $\log_{10}$ CFU mL^{-1}) exceeded 2 log CFU g^{-1} and 1-2 log CFU mL^{-1}, the set standards for fresh fish and fresh water respectively. Finally, while the mean SAC for the pond water was 4.09 $\log_{10}$ CFU mL^{-1} that of the fish skin ranged from 4.2 to 4.7 $\log_{10}$ CFU g^{-1}. The pond sediment slightly registered higher microbial load (3.56-7.08 $\log_{10}$ CFU g^{-1}) compared to pond water (3.24-5.75 $\log_{10}$ CFU mL^{-1}). Interestingly, the mean TCC and faecal coliform count of fish meal were 2.6 $\log_{10}$ CFU g^{-1} and 2.13 $\log_{10}$ CFU g^{-1}, respectively.

3.2 Bacteria Isolation and Identification

Results in Fig. 1 show that *S. aureus* had the highest percentage occurrence (21.4%), followed by *Enterobacter sakazakii* (19%), *E. coli* (12%), *Citrobacter freundii* and *Serratia fonticola* (7%), *Klebsiella oxytoca* and *Proteus* spp. (4.7%) while *Aeromonas hydrophila*, *Kluyvera* spp., *Moraxella* spp., *Pasteurella multocida* and *Pseudomonas fluorescens* were the least frequent (2.4%).

Table 1 Microbial load of samples in the fish ponds at the Mfou fish farm.

| Sample	Total viable aerobic bacteria count ($\log_{10}$ CFU g^{-1})			Fungal count ($\log_{10}$ CFU g^{-1})			Total coliforms count ($\log_{10}$ CFU g^{-1})			Faecal coliforms count ($\log_{10}$ CFU g^{-1})			*Staphylococcus aureus* count ($\log_{10}$ CFU g^{-1})		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
Catfish intestine	6.93	7.0	7.15	ND	6.46	5.82	7.7	6.6	6.7	6.6	6.0	4.0	3.64	4.27	4.72
Catfish gills	6.51	6.69	5.59	ND	5.19	4.31	6.2	6.0	4.6	6.4	4.2	3.9	3.94	5.18	3.97
Catfish skin	5.40	5.56	5.45	ND	4.25	3.93	4.6	5.1	4.4	4.6	4.0	3.1	4.28	5.76	4.03
Kanga intestine	7.17	6.40	6.70	ND	5.30	6.58	6.6	6.6	6.0	7.4	3.4	6.0	5.31	5.17	7.14
Kanga gills	6.51	6.48	6.92	2.00	4.54	4.98	5.2	4.4	6.0	6.0	3.6	4.0	4.60	3.69	3.81
Kanga skin	5.30	6.48	8.10	ND	4.07	4.23	4.4	4.3	4.7	4.0	3.6	3.8	4.78	4.00	3.82
Tilapia intestine	6.98	5.00	5.35	2.54	4.56	4.73	5.6	4.6	5.4	5.4	4.6	4.0	4.55	3.79	4.66
Tilapia gills	6.63	4.70	6.58	2.18	4.48	5.15	5.6	4.0	5.0	5.0	4.2	4.0	5.26	4.96	3.94
Tilapia skin	4.79	5.49	6.10	ND	4.00	5.54	3.6	5.2	6.4	4.0	3.4	3.6	4.32	4.72	4.24
Mud	5.94	8.49	6.81	ND	5.30	5.40	4.6	5.0	5.6	5.0	3.9	4.9	4.17	4.66	4.20
Water ($\log_{10}$	5.00	6.49	5.77	ND	4.26	5.36	5.0	5.2	5.2	4.0	3.0	4.3	3.38	4.95	3.94

CFU mL^{-1})

ND: not detected.

Table 2   Mean microbial load (log$_{10}$ CFU g^{-1} or mL^{-1}) of fish skin and fish pond environment at the Mfou fish farm.

Sample		TVC	FC	TCC	FCC	SAC
	Intestine	7.02	4.09	7.0	5.53	4.21
Catfish	Gills	6.26	3.16	5.6	4.83	5.03
	Skin	5.74	2.72	4.7	3.9	4.7
	Intestine	6.75	3.96	6.4	5.6	5.87
Kanga	Gills	6.63	3.84	5.2	4.53	4.03
	Skin	6.62	2.77	4.47	3.8	4.2
	Intestine	5.77	3.94	5.2	4.67	4.33
Tilapia	Gills	5.97	3.93	4.86	4.4	4.72
	Skin	5.46	3.18	5.06	3.7	4.42
Pond sediment	Mud	7.08	3.56	5.06	4.6	4.34
Pond water	Water	5.75	3.24	5.13	3.76	4.09

TVAC: total viable aerobic bacterial count; FC: fungal count; TCC: total coliform count; FCC: faecal coliform count; SAC: *S. aureus* count.
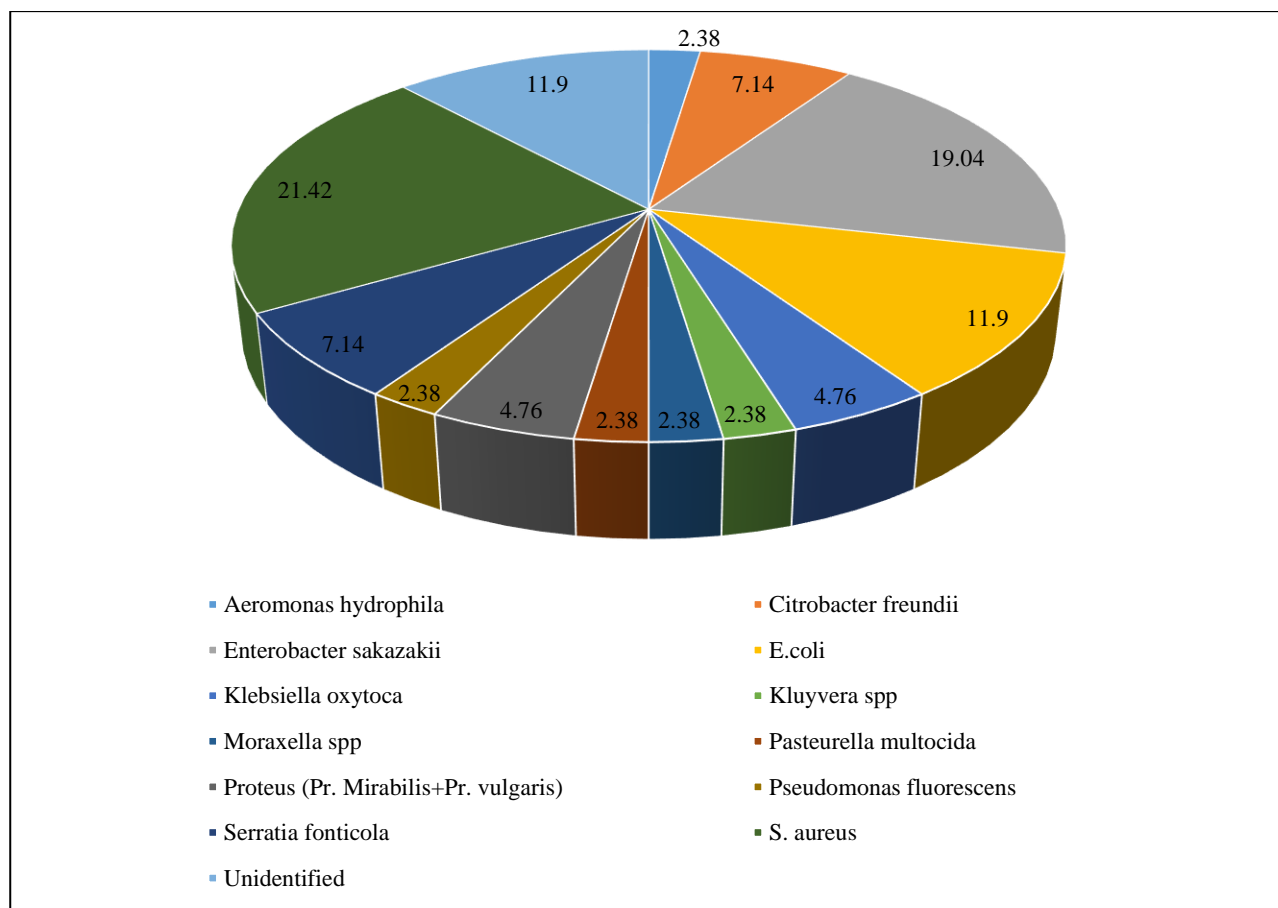
Fig. 1 Microbial diversity of fish skin from the Mfou fish farm.

3.3 Antimicrobial Susceptibility

Though more than 97% of bacteria were susceptible to chloramphenicol, 96% and 100% of them were resistant to penicillin G and erythromycin, respectively (Table 3). Moreover, 52% of the tested bacteria showed resistance to sulfamethoxazole/trimethoprim

and tetracycline. All the studied bacteria had MAR index ranging from 0.4 to 1.0 (> 0.2). *E. coli* and *Proteus vulgaris* had the highest MAR index (1.0) while *S. aureus* registered the least MAR index (0.4).

Table 3 Antibiotic profile and MAR index of bacterial isolates from fish skin at the Mfou fish farm.

Code	Organisms	P10	C30	SXT25	E15	TE30	MAR index
CF EMB-1	*E. coli*	+	+	+	+	+	1
CF EMB-2	*E. coli*	+	+	+	+	+	1
CF EMB-3	*Klebsiella oxytoca*	+	-	+	+	+	0.8
CF EMB-4	*Klebsiella oxytoca*	+	-	+	+	+	0.8
CF MC-1	Unidentified	+	-	+	+	+	0.8
CF MC-2	*Proteus vulgaris*	+	+	+	+	+	1
CF MC-3	*Enterobacter sakazakii*	NT	NT	NT	NT	NT	NA
CF MC-4	*Serratia fonticola*	NT	NT	NT	NT	NT	NA
CF-MSA-1	*S. aureus*	NT	NT	NT	NT	NT	NA
CF-MSA-2	*S. aureus*	NT	NT	NT	NT	NT	NA
CF-MSA-3	*S. aureus*	NT	NT	NT	NT	NT	NA
CF SS-1	*Serratia fonticola*	+	-	-	+	-	0.4
CF SS-2	*Proteus mirabilis*	+	-	+	+	+	0.8
CF SS-3	*Serratia fonticola*	+	-	-	+	-	0.4
CF SS-4	Unknown	NT	NT	NT	NT	NT	NA
CF SS-5	Unknown	NT	NT	NT	NT	NT	NA
CF TCBS-1	*E. coli*	NT	NT	NT	NT	NT	NA
CF TCBS-2	*Moraxella* spp.	NT	NT	NT	NT	NT	NA
KF MC-1	*Citrobacter freundii*	+	-	+	+	+	0.6
KF MC-2	*Enterobacter sakazakii*	NT	NT	NT	NT	NT	NA
KF MC-3	*Citrobacter freundii*	+	+	+	+	-	0.8
KF MC-4	*E. coli*	+	+	+	+	-	0.8
KF MC-5	*Enterobacter sakazakii*	+	-	-	+	-	0.4
KF MC-6	*Enterobacter sakazakii*	+	-	-	+	-	0.4
KF-MSA-1	*S. aureus*	+	-	-	+	-	0.4
KF-MSA-2	*S. aureus*	NT	NT	NT	NT	NT	NA
KF-MSA-3	*S. aureus*	NT	NT	NT	NT	NT	NA
KF SS-1	*Enterobacter sakazakii*	+	-	-	+	-	0.4
KF SS-2	*Enterobacter sakazakii*	+	-	-	+	-	0.4
KF TCBS-1	*Pseudomonas fluorescens*	NT	NT	NT	NT	NT	NA
KF TCBS-2	*Pasteurella multiccida*	NT	NT	NT	NT	NT	NA
TF MC-1	*Citrobacter freundii*	+	-	-	+	-	0.4
TF MC-2	*Enterobacter sakazakii*	+	-	-	+	-	0.4
TF MC-3	*Enterobacter sakazakii*	+	-	-	+	-	0.4
TF-MSA-1	*S. aureus*	+	-	-	+	+	0.6
TF-MSA-2	*S. aureus*	+	-	-	+	-	0.4
TF-MSA-3	*S. aureus*	+	-	+	+	+	0.8
TF SS-1	Unidentified	+	+	+	+	+	1
TF SS-2	*Aeromonas hydrophila*	-	+	-	+	+	0.6
TF TCBS-1	*E. coli*	+	-	+	+	+	0.8
TF TCBS-2	*Kluyvera* spp.	+	-	+	+	+	0.8
TF TCBS-3	Unidentified	NT	NT	NT	NT	NT	NT
No. of isolates tested		27	27	27	27	27	
No. of resistant isolates		26	7	14	27	14	

Percentage of resistant isolates (%)	96.3	2.6	51.8	100	51.8

Code—CF: Catfish skin, KF: Kanga fish skin, TF: Tilapia fish skin.

Symbol—+: resistant, -: susceptible, NT: not tested, P10: penicillin, C30: chloramphenicol, SXT25: sulfamethoxazole/trimethoprim, E15: erythromycin, TE: tetracycline, MAR index: Multiple Antibiotic Resistance index.

4. Discussion

4.1 Significance of Microbial Contamination of Mfou Aquaculture Site

The fish production farm at Mfou appears to be highly contaminated. It is very interesting to note that the TVAC of fish irrespective of the parts and species was beyond the acceptable level (5.00 $\log_{10}$ CFU g^{-1}) as defined by the International Commission on Microbiological Specifications for Foods [11]. The TVAC is used as a quality indicator. Its maximum microbiological limit is set to separate the good quality fish products from the bad ones [11]. The mean TVAC of pond fish and pond water in the present study were higher than those reported by previous studies in Nigeria and Sudan [4, 12, 13]. Equally, the mean TCC and FCC of pond water and fish at the Mfou fish production farm were beyond the recommended limits set at 1-2 $\log_{10}$ CFU mL^{-1} and 2 $\log_{10}$ CFU g^{-1} respectively, for fresh water and fresh fish [14]. The high counts of TCC and FCC in the present study are evidence for fecal contamination of the aquaculture. The close proximity of the ponds to the residential areas, the practice of integrated fish farming system with poultry, goat, and the substantial contamination of fish meal were critical contributing factors for the high levels of microorganisms in the fish ponds. As it is common with the integrated fish farming practices [15], faecal or organic contaminants from residential areas via pet droppings or sewage wastes might have found their ways into the aquaculture site. Particularly, the heavy bacterial load and specifically, the high thermo-tolerant coliforms count in this study could compromise the quality of the pond water. This poor bacteriological quality of the pond water appeals to fish producers not only because of its human health risk potentials, but also because of its associated serious setback effect on

achieving and maintaining viable aquaculture production [14]. Therefore, there is urgent need to monitor the bacterial load and quality of the fish ponds at the Mfou aquaculture production farm. Moreover, the international standard defined by European Union Commission [16] recommends SAC of fresh fish not to exceed 2 $\log_{10}$ CFU g^{-1}. Unfortunately, the fish samples in the current study recorded higher levels of SAC than the standard. Our results corroborate with the findings of Sichewo and colleagues [17] who found in the edible fish from an aquaculture site in Zimbabwe higher staphylococcal count than the acceptable limit. However, the staphylococcal load of the intestines of the fish species studied previously in fish ponds in the western region of Cameroon was slightly higher than our findings [1].

4.2 Bacteria Isolation and Identification

The bacterial load of pond water in the present study could have contributed to the high microbial diversity of fish given that pond water quality reflects the microflora of the aquaculture fish [3]. Isolation and identification of enteric bacteria (*E. coli*, *P. mirabilis*, *P. vulgaris*, *Klebsiella oxytoca*, *Citrobacter freundii*, and *E. sakazakii*), and other bacterial species namely *S. aureus*, and *Pseudomonas fluorescens* indicate multisource pollution of fish notably the sewage effluents and animal and agricultural wastes. *S. aureus* was the most abundant bacterial isolate in the Mfou aquaculture site. This result agrees with the finding of Sichewo and teammates who equally reported *S. aureus* as the most abundant bacterial species in a rural Zimbabwean aquaculture [17]. Humans are the main source of *S. aureus* and its presence in the aquaculture environment may be orchestrated by anthropogenic activities through improper hygienic and sanitary conditions. However, *Enterobacter sakazakii* was the most abundant

Gram-negative bacteria followed by *E. coli*. The isolation of *Aeromonas hydrophila,* a well-known fish pathogen, is of paramount importance as it is associated with mass mortality in aquaculture targeting particularly tilapia species [18, 19]. Its pathogenicity is attributed to several virulence factors explaining its high mortality rate in fish [20]. In addition, its zoonotic status makes it an important human pathogen due to its involvement in human diarrhoeal illnesses [19]. The presence of *Pasteurella multocida* in the fish skin is of public health importance as it has been involved in fish infection [20] and human cases of meningitis [21]. Though, *Moraxella* sp. is an opportunistic bacterial pathogen, it has also been cited as a threat to "stressed" fish [22]. The identification of *Serratia fonticola*, involved in community-acquired urinary tract infection is equally of public health relevance [23, 24]. Other fish pathogen isolates in this study were *Citrobacter freundii*, and *Pseudomonas fluorescens* [23, 25]. The isolation of *E. coli* and *S. aureus* in fish constitute another potential health risk for the consumers, especially those who eat raw or insufficiently cooked fish. The presence in high numbers of fecal coliforms unveils high risk of foodborne disease outbreaks [26]. With the exception of *Serratia fonticola*, *Moraxella* spp. and *Kluyvera* spp. (only detected in the present study), the microbial diversity of the aquaculture in Mfou is similar to what was previously described by Akoachere and colleagues [27] from the coastal waters in South West Cameroon.

4.3 Antibiotic Resistance Profile of Bacterial Isolates

The phenotypic antimicrobial sensitivity pattern of fish bacterial isolates indicates that chloramphenicol is still an efficient antibiotic in Cameroonian aquaculture as it retained antibacterial activity against 97% bacteria isolates. However, despite this high performance in the present study, chloramphenicol is not authorized for use in food producing animals in the European Union and many developed countries

owing to its dangerous side effects [28]. Even though this drug is not legally banned in the Cameroonian veterinary sector, chloramphenicol is less preferred to florfenicol and is being gradually substituted by thiamphenicol in clinical settings [29, 30]. Therefore, its strong antibacterial activity may be attributed to its less usage. On the other, the current study indicates that penicillin G and erythromycin, useful for the treatment of Gram-negative bacteria are no longer recommendable. It should be noted that erythromycin is listed among the drugs that are usually overdosed by farmers in Cameroon [5].

Generally, the high prevalence of antibiotic resistance among bacterial isolates from the fish skin may be attributed to the bioaccumulation of antibiotic residues in fish meal or pond sediment. It is also possible that antibiotics from the fish meal could have been absorbed into the sediment causing bacterial selection in the aquaculture environment [31]. In fact, previous studies reported the presence of antibiotic residues in the fish muscles due to the use of antimicrobials for the prevention and treatment of bacterial infections in aquaculture [32-34]. The run-off of animal droppings or spilling of chicken litter manure containing antibiotic residues [6] during raining season could also be mentioned among key drivers of multidrug resistance development in this study. Moreover, the non-referral of most fish farmers (75%) in Cameroon to a veterinarian for prescription [2] could be an important contributing factor to the high antibiotic resistance profile of bacterial isolates in the current study. On the other hand, these resistant bacteria could find their ways into the environment during the frequent discharging of pond wastewater. In return, the discharge of wastewater may promote the introduction also resistant non-pathogenic bacteria in the human environment with potential horizontal transfer of antimicrobial resistance genes. It is alarming to indicate that all the bacterial isolates in this research work were multiresistant to various antibiotics with MAR index greater than 0.2. This

suggests that all the isolates came from high antibiotic usage area. Microbial multidrug resistance frustrates efforts for disease control resulting in a prolonged hospitalization of patients [4]. Some of the antibiotics including sulfonamides, tetracycline, erythromycin, chloramphenicol to which bacteria were resistant are either critically or highly important antimicrobials [35]. Moreover, all these drugs are frequently used in both veterinary and human medicine in Cameroon [29, 30].

5. Conclusion

The microbial load of samples (fish parts, mud and pond water) generally exceeded the acceptable levels in terms of TVAC, TCC, FCC and SAC. Our study has shown that fish are sources of fish and human pathogenic bacteria. Curiously, all these bacteria exhibited high resistance against the studied drugs except chloramphenicol. This finding is of clinical and epidemiological relevance suggesting the application of strict hygiene measures during handling, processing, and consumption of fish cultured at the Mfou aquaculture site. Sterilization of fish meal and manure should also be undertaken to minimize microbial contamination. Good quality water such as borehole should be used in the fish pond rather than water from stream and swamp waters. Waste water from the fish pond should equally be treated before disposal into the surrounding environment. Antibiotic susceptibility pattern of the bacterial isolates should also be monitored constantly to predict the emergence and widespread of MAR. Integrated fish farming in this area could be considered as a reservoir for the antibiotic resistant bacteria.

Further research is needed to elucidate the microbial diversity of the entire aquaculture (pond water sediment, feeds, and fish parts) and evaluate the seasonal variation impact on the microbial and physico-chemical quality of the Mfou aquaculture production site. It is also important in future to investigate the possible causes of the high antibiotic resistance observed in the present study. In view to boosting local fish production in Cameroon, it is imperative to extend this research work to all the aquaculture farms in the country.

Conflict of Interests

The authors declare no conflicts of interest.

Acknowledgments

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