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Bacterial Microflora in the Gut, Gill and Skin of African Catfish, C*larias garriepinus* (Burchell, 1822) collected from Earthen Ponds in Oke-Baale, Osogbo, Nigeria.

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ABSTRACT: *The African catfish is a widely cultivated fish species in Nigeria, as it serves as a good source of nutritive protein. The bacterial microflora in the different tissues of African catfish (Clarias gariepinus) in earthen ponds located in Oke-Baale, Osogbo, Nigeria were investigated. Fish obtained were microbiologically examined for the bacterial isolates in the gut, gill and skin tissues.. Bacterial isolates such as Coagulase-Negative Staphylococci (CoNS), Staphylococcus aureus, Streptococcus spp, Corynebacterium spp and Aeromonas spp were isolated from the tissues of the cultured fish. The results of this study showed that fish cultivated in earthen ponds harbor a host of bacteria that may be harmful to man if proper food hygienic practices are not adopted before consumption and also the need for healthy aquacultural practices by fish farmers so as to avoid zoonotic infection.*

Keywords: African catfish; earthen ponds; bacterial microflora; fish tissues; aquaculture

Introduction

The African catfish, *Clarias gariepinus* is a prominent cultivable fish species in Nigeria, and of course in many African countries. It provides a rich and cheap protein source to many poor families that could not afford other protein sources [1]. This fish species is highly tolerant of extreme environmental conditions and it is quite easy to cultivate [2]. These factors combined with its high quality flesh, distinctive taste and texture, relatively low fat and absence of intramuscular spines makes *Clarias* the most cultivable species compared to other fish species [3, 4].

Various aquacultural practices, such as concrete ponds, barrage ponds, parallel ponds, earthen ponds etc. are being employed in the cultivation of *Clarias species*. Of these pond systems, most local farmers with little financial resources often prefer the earthen pond system because its maintenance is relatively cheaper compared to other systems [5]. However, when earthen ponds are employed for fish cultivation, fertilizers are often applied to improve fish yield, but because this always come with huge financial implication, local fish farmers prefer alternative nutrients enrichment methods such as the use of animal manure. While these alternatives may save cost, studies have shown that such practices can increase microbial loads on the resident fish [6].

Some farmers routinely use dead animals to feed fish in earthen ponds. This practice may also result in increased microbial load, and encourage growth and development of planktonic and benthic organisms, which may seriously affect the water quality. Some farmers make regular application at three or four months intervals. Others depend on visual observation and add the waste according to the colour of the pond. Aside the use of animal manure and feeding fish with dead animals, one important factor that has been reported to result in bacterial infection of fish in earthen pond is overpopulation [7]. Many local farmers in an attempt to reduce cost usually stock their ponds with high densities of fish, which favours rapid spread of infectious bacteria and acute outbreaks of diseases.

While studies have reported bacterial infections on most cultivable fish species e.g. Tilapia [6], the Brazilian paku [8], rainbow trout [9] etc., there are relatively few information on bacterial microflora in cultivated *Clarias* [10], and to the best of our understanding no study have attempted to link bacterial infection in *Clarias* to a particular aquacultural practice. This study was therefore undertaken to investigate the diversity of bacterial populations in different tissues of fish that are grown in earthen ponds in Oke-Baale, Osogbo, Nigeria.

Materials and Methods

Collection and Preparation of Samples

Adult catfish (n=5) of average weight 300 ±10g were collected from five different earthen ponds in a local, commercial fish hatchery located in Oke-Baale, Osogbo, Nigeria (07ę43'55N; 004ę31'07E). They were then transported live in plastic containers to the Microbiology Laboratory of the Department of Biological Sciences of the Osun State University, Osogbo, Nigeria. Fish were anaethesized with benzocaine and then dissected. About 1g of the skin, gill and gut of each fish was introduced in nutrient broth for isolation and identification of bacterial microflora in the skin, while normal saline was used for bacterial isolation and identification in the gut and gill.

Isolation, Morphological and Biochemical Characterization of Bacteria

Aliquots of 1ml fish samples were taken using a sterile pipette and cultured in nutrient agar using pour plate method. The plates were incubated at 37ºC for 24 hours. The plates were observed for growth after 24 hours and further sub culturing was done to obtain pure isolates. The colonial morphologies of the pure isolates obtained were observed. The pure isolates were stored on nutrient agar slant at 4ºC in preparation for morphological and biochemical characterization. The characterization of bacterial isolates was based on Gram stain reaction, catalase test, indole production, citrate test, starch hydrolysis, DNase test, coagulase test and sugar fermentation test. The morphological and biochemical tests performed on the isolates followed the procedures reported by Finegold and Baron [11] and Prescott et al. [12].

Table 1: Synopsis of bacterial isolates from the different tissues of *Clarias gariepinus* obtained from earthen ponds in Oshogbo, Nigeria. GRAM-Gram reaction, CAT- Catalase test, COAG- Coagulase test, DNase- DNase test, INDO-Indole test, STA-Starch test, CIT- Citrate test, SUC- Sucrose, MAN- Mannitol, GLU-Glucose, FRU-Fructose, LAC-Lactose, + Positive, ï Negative, $+^{G}$ Positive with gas production, ND- Not determined

CODE	SOURCE	GRAM	CAT	COAG	DNase	INDO	STA	CIT	SUC	MAN	GLU	FRU	LAC	ISOLATE
A1	GUT	+ cocci	+	ï	ï	ND	ND	+	+	$+^{G}$	$+^{G}$	$+^{G}$	$+^{G}$	CoNS (*S. xylosus*)
A2	GUT	+ cocci	+	ï	+	ND	ND	+	+	ï	$+^{G}$	$+^{G}$	$+^{G}$	CoNS (*S. epidermidis*)
A3	GUT	+ cocci	+	ï	+	ND	ND	+	+	ï	ï	ï	ï	CoNS
A4	GUT	+ cocci	+	ï	ï	ND	ND	+	+	+	ï	+	ï	CoNS
A5	GILL	+ cocci	+	ï	ï	ND	ND	ï	ï	ï	ï	ï	ï	CoNS
A6	GILL	+ cocci	+	ï	ï	ND	ND	ï	+	$+^{G}$	$+^{G}$	$+^{G}$	+	CoNS (*S. xylosus*)
A7	GILL	+cocci	+	ï	ï	ND	ND	+	ï	$+^{G}$	ï	+	+	CoNS(*S. simulans*)
A8	SKIN	+cocci	+	ï	ï	ND	ND	+	+	$+^{G}$	$+^{G}$	$+^{G}$	+	CoNS (*S. xylosus*)
A9	SKIN	+cocci	+	ï	+	ND	ND	+	$+^{G}$	ï	$+^{G}$	+	$+^{G}$	CoNS (*S. epidermidis*)
A10	GILL	+cocci	+	+	+	ND	ND	+	ï	+	+	+	ï	*Staphylococcus aureus*
A11	GUT	+cocci	+	+	+	ND	ND	+	$+^{G}$	$+^{G}$	$+^{G}$	+	$+^{G}$	*Staphylococcus aureus*
A12	GILL	+cocci	+	+	+	ND	ND	+	ï	ï	ï	ï	ï	*Staphylococcus aureus*
A13	GUT	+cocci	+	+	+	ND	ND	ï	ï	ï	ï	ï	ï	*Staphylococcus aureus*
A14	GUT	+cocci	ï	ND	ND	ND	ND	ï	ï	ï	ï	ï	ï	*Streptococcus spp*
A15	GILL	+cocci	ï	ND	ï	ND	ND	ï	ï	$+^{G}$	ï	ï	ï	*Streptococcus spp*
A16	GUT	+cocci	ï	ND	ï	ND	ND	ï	ï	+	ï	ï	ï	*Streptococcus spp*
A17	GUT	+rod	+	+	ND	ND	+	+	ï	ï	ï	ï	ï	*Corynebacterium spp*
A18	GILL	+rod	+	+	+	ND	+	+	ï	+	ï	ï	ï	*Corynebacterium spp*
A19	GILL	+rod	+	+	ND	ND	ï	ï	ï	+	ï	ï	ï	*Corynebacterium spp*
A20	SKIN	ï rod	_	ND	ND	ï	ï	+	ï	$+^{G}$	+	$+^{G}$	+	*Aeromonas spp.*

Table 2: Frequency of occurrence of bacteria in the different tissues of *C. gariepinus*

ISOLATES	FREQUENCY (%)
Coagulase Negative Staphylococci (CoNS)	9 (45)
Staphylococcus epidermidis	2 (22.22)
Staphylococcus xylosus	3 (33.33)
Staphylococcus simulans	1 (11.11)
Others	3 (33.33)
Staphylococcus aureus	4 (20)
Streptococcus spp	3 (15)
Corynebacterium spp	3 (15)
Aeromonas spp	1 (05)
Total	20 (100)

Results and Discussion

The results of this study show that catfish that were raised in earthen ponds do harbor a huge bacterial microflora of pathogenic and/or spoilage importance. The bacterial populations isolated and identified in this study include *Staphylococcus epidermidis, S. xylosus, S. simulans, S. aureus, Streptococcus spp, Corynebacterium spp* and *Aeromonas spp*. Of all the bacterial genera identified, the Coagulase-Negative Staphylococci (*Staphylococcus epidermidis, S. xylosus, S. simulans*) were the most prominent in all the fishes, occurring 9 times out of 20 times while *S. aureus* followed in occurrence frequency occurring 4 times out of 20, *Streptococcus spp* and *Corynebacterium spp* both occurred 3 times out of 20. The frequency of occurrence was least in *Aeromonas spp* occurring only 1 time out of 20 (Tables 1 & 2).

The higher frequency of occurrence of Coagulase-Negative Staphylococci (CoNS) compared to *S. aureus*, in this study is consistent with reports from clinical isolates data that showed that the CoNS are more common pathogens than *S. aureus* [13]. The CoNS is long thought of to be a group of non-pathogenic bacteria but recent reports indicated the potential for these groups of bacteria to being pathogenic. The CoNS have been reported to result in bacteraemia (presence of viable bacteria in the blood stream), which is usually not life-threatening but in immunocompromised patients, frank sepsis syndrome may occur which is quite fatal and with a high rate of mortality [14]. The relative higher occurrence of *S. aureus* in this study is not uncommon. There have been reports of heavy infestation of cultivated fish species with this bacterium, especially in earthen ponds [6]. The fact that *S. aureus* is quite abundant in fish tissues necessitates the need to properly boil these fish before consumption. This will help to prevent the risk of staphylococcal food poisoning, a very dangerous infection caused by consumption of the exotoxins produced by this bacterium [15]. *Streptococcus spp* have been widely isolated in the tissues of different fish species such as rainbow trout, salmon, golden shiners, Eel, tilapia, sturgeon and striped bass [16, 17]. Fish infection with *Streptococcus spp* is very dangerous and has been reported to result in high mortality in infected fish. Streptococcal infections in fish are often diagnosed by erratic swimming behaviours as well as loss of buoyancy in infected fish [18]. In human, streptococcal infection resulted in cellulitis (infection of connective tissues) of the hand, and consequently resulting in fever in the patients [19]. This condition is associated with open wounds in fish handlers and mostly debilitating in immunocompromised individuals. The possibility of human getting infected with these bacteria through fish handling necessitated the need to observe healthy practices during fish handling such as wearing of gloves, especially in a situation of open wounds.

Two other bacteria genera that were isolated from the fish in this study are *Corynebacterium spp* and *Aeromonas spp*. *Corynebacterium spp* have been isolated in fish tisuues [20, 21]. Fish infected with *Corynebacterium spp* has been shown to exhibit bilateral exophthalmia, bulging of the eye anteriorly out of the orbit [20]. The infection of cultivated fish species with *Aeromonas spp* has been extensively reported in the literature [22, 23]. The motile representatives of *Aeromonas spp.* e. g. *A. salmonicida* and *A. hydrophila* cause diseases like the hemorrhagic septicemia, red sore diseases, furunculosis and ulcer in fish [24, 25], and thus leading to high economic losses in the fishery farms.

This study has provided evidence that fish cultivation in the earthen ponds might result in their harboring of quite a great population of bacterial microflora. In most developing countries where substantial portion of the populace depends on fish as a source of dietary protein, careful attention must be given to proper fish processing prior to consumption to avoid the risk of food poisoning. Since there is the possibility of zoonotic infection of human by most of the bacteria isolated from the different tissues of these fish, local fish farmers should be properly educated on the need to maintain a good and healthy aquacultural practices such as wearing of gloves, and also making deliberate efforts to reduce contacts with cultivated fish during fish handling and maintenance.

References

1. USAID MARKETS: Best Management Practices for Fish Farmers in Nigeria. 38 pp. 2010.
2. Luckhoff, PD: Application of the condition factor in the production of African sharptooth catfish, Clarias gariepinus. Unpublished M.Sc. Thesis, University of Stellenbosch, South Africa. 62pp. 2005.
3. El-Shebly, AA: Evaluation of growth performance, production and nutritive value of the African catfish, C*larias gariepinus* cultured in earthen ponds. Egyptian Journal of Aquatic Biology and Fisheries 10 (3): 55-67. 2006.
4. Khwuanjai, H, Ward, FJ, Pornchai, J: The effect of stocking density on yield, growth and mortality of African catfish (*Clarias gariepinus,* Burchell 1822) cultured in cages. Aquaculture 152: 67-76. 1997.
5. Ugwumba, COA, Okoh, RN: African Clariid catfish farming in concrete and earthen ponds: A comparative profitability analysis. Journal of Fisheries International 5(1): 14-18. 2010.
6. Ampofo, JA, Clerk, G: Diversity of bacteria contaminant in tissue of a fish cultured in organic waste fertilized ponds. Open Fish Science Journal 3: 142-146. 2010.
7. Balasubraman, S, Rajan, MR, Raj, SP: Microbiology of fish grown in a sewage-fish pond. Bioresource Technology 40: 63-66. 1992.
8. Carraschi, SP, Cruz, C, Neto, JG, Moraes, FR, Júnior, OD, Neto, AN, Bortoluzzi, NL: Evaluation of experimental infection with *Aeromonas hydrophila* in pacu (*Piaractus mesopotamicus*) (Holmberg, 1887). International Journal of Fisheries and Aquaculture 4(5): 81-84. 2012.
9. Cipriano, RC, Holt, RA: *Flavobacterium psychrophilum*, cause of Bacterial Cold-Water Disease and Rainbow Trout Fry Syndrome. Fish Disease Leaflet No. 86. United States Dept. of the Interior. U.S. Geological Service, National Fish Health Research Laboratory, Kearneysville, WV. 44pp. 2005.
10. Oladosu-Ajayi, RN, George, FOA, Obasa, SO, Ajayi, AA, Bankole, MO: Bacterial load, composition and succession in the African catfish, *Clarias gariepinus* (Burchell, 1822) held at ambient temperatures. Researcher 3: 67-73. 2011.
11. Finegold, SM, Baron, EJ: Methods for identification of etiologic agents of infectious disease. In: Bailey and Scottôs Diagnostic Microbiology.7th edition. 1986.
12. Prescott, LM, Harley, JP, Klein, DA: Microbiology. New-York , McGraw-Hill Companies, Inc. 2005.
13. Eiff, C, Peters, G, Heilmann, C: Pathogenesis of infections due to Coagulase-Negative Staphylococci. Lancet Infectious Diseases 2: 677-685. 2002.
14. Pagano, L, Taconelli, E, Tumbarello, M: Bacteraemia in patients with hematological malignancies. Analysis of risk factors, etiological agents and prognostic indicators. Haematologica 82: 415-419. 1997.
15. Richards, MS, Rittman, M, Gilbert, TT, Opal, SM, DeBuono, BA, Neill, RJ, Gemski, P: Investigations of a staphylococcal food poisoning outbreak in a centralized school lunch program. Public Health Reports 108: 765-771. 1993.
16. Hoshina, T, Sano, T, Morimoto, Y: A *Streptococcus* pathogenic to fish. Journal of Tokyo University of Fisheries 44: 57-68. 1958.
17. Inglis, V, Roberts, RJ, Bromage , NR: Streptococcal infections. In: Bacterial Diseases of Fish, Halsted Press, John Wiley & Sons, Inc., NY. 196-97. 1993.

18. Yanong, RPE, Francis-Floyd, R: Streptococcal infections of fish. Institute of Food and Agricultural Sciences, University of Florida, Circular 57, 6pp. 2002.
19. Swartz, MN: Cellulitis. The New England Journal of Medicine 350: 904-912. 2004.
20. Baya, AM, Lupiani, B, Bandin, I, Hetrick, FM, Figueras, A, Carnahan, A, May, EM, Toranzo, AE: Phenotypic and pathobiological properties of *Corynebacterium aquaticum* isolated from diseased striped bass. Diseases of Aquatic Organisms 14: 115-126. 1992.
21. Cahill, MM: Bacterial flora of fishes: a review. Microbial Ecology 19: 21-41. 1990.
22. Orozova, P, Chikova, V, Najdenski, H: (2010). Antibiotic resistance of pathogenic for fish isolates of *Aeromonas* spp. Bulgarian Journal of Agricultural Sciences 16: 376-386. 2010.
23. Kamble, SR, Meshram, SU, Shanware, AS: Characterisation of *Aeromonas* species isolated from diseased fish using ERIC- RAPD Markers. Asian Pacific Journal of Molecular Biology and Biotechnology 20(3): 99-106. 2012.
24. Austin, B, Adams, C: Fish pathogens. In: Austin, B. M., Altwegg, M., Gosling, P. J., Joseph, S .(Eds). The Genus *Aeromonas*, Wiley, Chichester, UK, 198ï 243. 1996.
25. Igbinosa, IH, Igumbor, EU, Aghdasi, F, Tom, M, Okoh, AI: Emerging *Aeromonas* species infections and their significance in public health. The Scientific World Journal Article ID 625023, 13 pp. 2012.