BACTERIOLOGICAL ANALYSIS OF CATFISH (*Clarias gariepinus*) FROM EARTHEN AND CONCRETE PONDS OF A REPUTABLE FISH FARM IN MAKURDI, BENUE STATE, NIGERIA

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Abstract

Bacteriological analysis of catfish (*Clarias gariepinus*) from earthen and concrete ponds of a reputable Fish Farm, Makudi, Benue State was investigated. The gills (7.8×10^6), Alimentary canal (8.2×10^6), fins (4.6×10^5) and skin (3.2×10^5) of *C. gariepinus* from earthen ponds had higher viable count than the gills (5.2×10^6) Alimentary canal (6.6×10^6), fins (2.3×10^5) and skin (2.6×10^5) of *C. gariepinus* from the concrete pond. Also coliform count of gills ((6.3×10^5) alimentary canal (6.7×10^5), fin (3.2×10^4) and skin (3.4×10^4) of *C. gariepinus* from earthen pond was higher than the coliform counts of gills (3.1×10^5), alimentary canals (3.6×10^4), fins (1.8×10^3) and skin (2.1×10^3) of *C. gariepinus* from concrete pond. Different species of bacteria with different shapes and morphology ranging from *Staphylococcus sp, Escherichia coli, salmonella typhi, sligellasp, enterobactersp, klebsiellasp, psuedomonasssp, bacillus sp,* to *enterococcus faecalis* were observed. These organisms were variously present in/on the different body parts of *C. gariepinus* from the earthen and concrete ponds. Of the various body parts of *C. gariepinus* from the concrete and earthen ponds, highest viable count (6.6×10^6) for *C. gariepinus* from the concrete and (8.2×10^6) from the earthen pond were recorded for alimentary canal while the lowest (2.3×10^5) for *C. gariepinus* from the concrete and (3.2×10^5) from the earthen pond were recorded for fins and skin, respectively. In addition, of the various body parts of *C. gariepinus* from the concrete and earthen ponds, highest coliform count (3.6×10^4) for *C. gariepinus* from the concrete and (6.7×10^5) from the earthen pond were recorded for alimentary canal while the lowest (2.3×10^5) from the concrete and (3.2×10^5) from the earthen pond were recorded for alimentary canal, however, the lowest coliform count (1.8×10^5) for *C. gariepinus* from the concrete and (3.2×10^4)from the earthen pond were recorded for fins, respectively. Generally, bacteria were more associated with *C. gariepinus* from the earthen pond than the concrete pond.

Keywords: *Clarias gariepinus*, Gills, fins, Alimentary canal, skin Earthen and Concrete Ponds

Introduction

Operations of aquaculture have produced over 66 million tonnes of food fish accounting for about $140 billion US dollars in 2012, which is 14 times higher compared to that of 1980 (FAO, 2014). Nevertheless disease stands an important limiting factor to the growth of the aquaculture industry (Bondad-Reantaso *et al.*, 2005) and accounts for economic losses of billions of dollars each year (Subasighe *et al.*, 2001).

Microbial communities, known as microbiota, play a vital function in maintaining host health through increasing digestion efficiency and utilisation of nutrients, boosting the immune system, and preventing attachment and increasing in number of opportunistic pathogens (Llewellyn *et al*., 2014). Interest in the manipulation of microbiota to gain advantage of these benefits and to prevent disease in aquaculture has increased tremendously (Sihag and Surma, 2012, Defordt *et al*., 2007). Nonetheless, in many fish species, the composition of the natural microbiota has not been characterized and as a result, the dominant bacterial players and their downstream influence on fish health are unclear.

Documenting the bacteria present in uninfected or healthy fish is an important first step to understanding the effects of microbial manipulation in aquaculture systems. With regards to disease resistance, the microbiota associated with gill and alimentary canal is of specific concern as these are primary and major routes for opportunistic pathogens to gain entry into fishes (Ringo *et al*., 2007). The bacterial abundance and diversity at these sites can provide insight into the health status of fish, as abundance of opportunistic pathogens increases and bacterial diversity decreases during stress and times of disease (Cipriano, 2011, Boutin *et al*., 2014). Due to the relevance of the microbiota in fish health and the interest in microbial manipulation to control diseases in aquaculture systems, this study aimed to thoroughly characterize microbiota associated with gill, alimentary canal, fins and skin of healthy *C. gariepinus*, a primary aquaculture candidate from earthen and concrete ponds of a reputable fish farm in Makurdi, Benue State.

Materials and Methods

Collection, Processing and Enrichment of Fish Samples

Ten samples of *Clarias gariepinus* of appreciable grams comprising of five samples each from earthen and concrete ponds of a reputable Fish Farm in Makudi, Benue State were purchased and taken to the Biological Science Department Laboratory, University of Agriculture, Makurdi for analysis. Through visual examination, the collected fish samples were confirmed to be all reasonably healthy. The gills, alimentary canals, fin and skin of the fish samples were aseptically obtained, minced, and grinded separately. 0.5 ml of each sample was then transferred to 4.5ml of 1% peptone and 10 fold serial dilutions were prepared for each of the samples.

Determination of Total Viable Count and Isolation of pure bacterial colonies

An aliquot of 0.5ml of each ten-fold diluted sample was inoculated unto prepared nutrient agar plates using spread-plate method. The plates were then incubated for 24-48 hours at 37^{o}_{C}. The number of colonies in each dilution was multiplied by the dilution factor to determine the total viable count.

An aliquot of each diluted sample was inoculated in nutrient broth at 37^{o}_{C} for 24 hours and isolation of bacteria was performed according to the method described by Egbebi *et al.*, (2016).

Identification and Characterization of bacterial isolates

Biochemical test and bacteria identification were performed on bacterial isolates in accordance with the method described by Cheesebrough (2006). All isolates were sub-cultured to obtain a pure culture after which a gram-staining was carried out

Determination of total Coliform Count

For each samples used for this study, two Eosin-Methylene Blue (EMB) agar plates were prepared. One was cultured with 100 L of the water sample while the second was cultured with 500 L of the water sample. All plates weree incubated at 37^{0}C for 48 hours. After the incubation period, the colonies were counted to assess the presence of fecal bacteria.

Results

Results of the coliform counts from the body parts of *Clarias gariepinus* from earthen and concrete ponds are shown in Table 1.

The gills (7.8×10^{6}), alimentary canal (8.2×10^{6}), fins (4.6×10^{5}) and skin (3.2×10^{5}) of *C. gariepinus* from earthen pons had higher viable count than the gills (5.2×10^{6}) Alimentary canals (6.6×10^{6}), fins (2.3×10^{5}) and skin (2.6×10^{5}) of *C. gariepinus* from the concrete pond. Also, coliform count of gills ((6.3×10^{5}) alimentary canals (6.7×10^{5}), fin (3.2×10^{4}) and skin (3.4×10^{4}) of *C. gariepinus* from earthen pond was higher than the coliform counts of gills (3.1×10^{5}), alimentary canals (3.6×10^{4}), fins (1.8×10^{3}) and skin (2.1×10^{3}) of *C. gariepinus* from concrete pond.

Table 1: Viable and Coliform count from the body parts of fish samples used for the study

Fish parts	Viable Count	Coliform Count
Gills		
Concrete Pond	5.2×10^6	3.1×10^5
Earthen Pond	7.8×10^6	6.3×10^5
Alimentary Canal		
Concrete Pond	6.6×10^6	3.6×10^4
Earthen Pond	8.2×10^6	6.7×10^5
Fins		
Concrete Pond	2.3×10^5	1.8×10^3
Earthen Pond	4.6×10^5	3.2×10^4
Skin		
Concrete Pond	2.6×10^5	2.1×10^3
Earthen Pond	3.2×10^5	3.4×10^4

Table 2 shows the results of cultural morphology and biochemical characteristics of bacteria isolates of *C. gariepinus* during the study period. Different species of bacteria with different shapes and morphology ranging from *Staphylococcus sp, Escherichia coli, salmonella typhi, sligellasp, enterobactersp, klebsiellasp, psuedomonasssp, bacillus sp,* to *enterococcus faecalis* were observed. These organisms were variously present in/on the different body parts of *C. gariepinus* from the earthen and concrete ponds. (Table 3)

Of the various body parts of *C. gariepinus* from the concrete and earthen ponds, highest viable count (6.6×10^6) for *C. gariepinus* from the concrete and (8.2×10^6) from the earthen pond were recorded for alimentary canal while the lowest (2.3×10^5) from the concrete and (3.2×10^5) from the earthen pond were recorded for fins and skin, respectively. In addition, of the various body parts of *C. gariepinus* from the concrete and earthen ponds, highest colliform count (3.6×10^4) for *C. gariepinus* from the concrete and (6.7×10^5) from the earthen pond were recorded for alimentary canal while the lowest (2.3×10^5) from the concrete and (3.2×10^5) from the earthen pond were recorded for alimentary canal, however, the lowest colliform count (1.8×10^5) from the concrete and (3.2×10^4) from the earthen pond were recorded for fins, respectively.

Generally, bacteria were more associated with *C. gariepinus* from earthen pond than the concrete pond.

Table 2. Cultural morphology and biochemical characteristics of bacteria isolates of *C. gariepinus*

Colony shape	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**	**Irregular**	**Circular**	**Irregular**	**Circular**
Colonu colour	Cream	Pink	Colourless	Colourless	Pink	Mucoid pink	Pale	Pale	Dark red
Morphology	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Grams reaction	+	-	-	-	-	-	-	+	+
Motility	-	+	+	-	+	+	+	-	-
Catalase	+	+	+	+	+	+	+	+	-
Citrate	-	-	+	-	+	+	+	-	-
Urease	-	-	-	-	-	+	-	-	-
Indole	-	+	-	-	-	-	-	-	-
Coagulase	+	NA	NA	NA	NA	NA	NA	NA	NA
MR	-	+	+	+	+	+	+	-	-
Oxidase	-	-	-	-	-	-	+	-	-
	Staphyloccocus sp	*Escherichia coli*	*Salmonella typhi*	*Shigella sp*	*Enterobacter sp*	*Klebsiella sp*	*Psuedomonas sp*	*Bacillus sp*	*Enterococcus faecalis*

Key: Na = Not applicable, + = Positive, - = Negative, MR = Methyl red

Table 3. Prevalence of the isolates of *C. gariepinus* from earthen and concrete ponds

Isolate	EPG	EPA	EPF	EPS	CPG	CPA	CPF	CPS
Staphyloccocus sp	+	+	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+	+
Salmonella typhi	-	-	-	+	-	+	-	-
Shigella specie	-	-	-	-	+	-	-	-
Enterobacter sp	+	+	-	-	+	-	+	-
Klebsiella sp	+	-	-	-	+	+	-	-
Psuedomonas spp	+	+	-	+	-	+	-	-
Bacillus sp	-	-	+	+	-	-	-	+
Enterococcus faecalis	-	-	+	-	+	-	-	-

KEY: EPG = Earthen Pond Gill, EPA = Earthen Pond Alimentary Canal, EPF = Earthen Pond Fin, EPS = Earthen Pond Skin, CPG = Concrete Pond Gill, CPA = Concrete Pond Alimentary Canal, CPF = Concrete Pond Fin, CPS = Concrete Pond Skin, + = Positive, - = NEGATIVE

Discussion

Different bacteria were isolated from ten samples of *C. gariepinus* purchased collected from both concrete and earthen fishponds of a reputable fish farm in Makurdi, Benue State, Nigeria. The gills, alimentary canals, fins and skin of the fish samples were investigated separately. The different species of bacteria isolated from all samples were *Staphylococcus spp., Escherichia spp., Salmonella typhi., Shigella sp., Enterococcus sp., Klebsiella sp., Pseudomonas sp., Bacillus sp.* and *Enterococcus faecalis.* These organisms were variously present in/on the different body parts of *C. gariepinus* from the earthen and concrete ponds. Isolation of these organisms from the concrete and earthen ponds in this work is not surprising as they have been previously isolated from the different body parts of *C. gariepinus* from different fishponds in Owo Area, Ondo State, Nigeria as reported by Egbebi *et al.,* (2016).

The higher load of bacteria associated with *C. gariepinus* from earthen pond may be due to contamination as a result of indiscriminate deposition of waste materias into the ponds through runoffs, animal excreta and other environmental wastes. During the rainy season, fecal matter as well as other forms of wastes from various sources is washed from contaminated land into River Benue which serves as the main source of water for fish culture in Benue State. Free roaming animals and pets such as dogs also contribute to fecal contamination of the pond. Besides that, stream and hold water used in earthen ponds and examining pools might be contaminated by coliform bacteria.

Variations in the bacterial load of gills, alimentary canals, fins and skin of the fish samples existed, being highest in the alimentary canals of the fish samples from both ponds but lowest on the skin.

The highest bacterial load encountered in the alimentary canal of the fish samples compared to the other body parts could be due large surface area provided by the alimentary canal and availability of different digested food particles present in the canal. This disagrees with the reported work of Egbebi *et al.,* (2016), Adebayo-Tayo *et al*., (2012) who reported highest bacterial load on the skin of *C. gariepinus* and attributed it to the constant exposure of the skin and its contact with the environment and its many pollutants.

The presence of *Salmonellaa typhi* in the alimentary canal of the fish samples is not surprising as it has been previously reported by Adedeji *et al*., (2011). Salmonellae tend to be associated with the skin, gills and intestines of catfish, but the most potential reservoir of Salmonella is the intestine (Adedeji *et al*., 2011).

The bacteria isolated included facultative pathogens which under stress, could give rise to disease of fish, and subsequently, to humans. *E. coli*, *Salmonella sp.*, and *Staphylococcus sp.* has been implicated in fish-borne diseases (Babu, 2000). Staphylococcus frequently causes septicemia, o*steomyelitis*, *bacteremia* and *otitis* (Udeze *et al.,* 2012). *Pseudomonas sp* could cause general inflammation and sepsis in critical body organs such as lungs, kidneys, urinary

tract, which can be fatal because it thrives in most surfaces (Udeze *et al.,* 2012). *E. coli* and *Shigella spp*. have been implicated for a number of gastroenteric diseases such as diarrhea, dysentery, vomiting, fever, colitis, hemolytic uremic syndrome with renal failure. *Salmonella sp* causes *salmonellosis*, which in humans could result in severe typhoid fever or salmonella fever and bacteremia (Egbere *et al.,* 2010). *Enterococcus sp* is a causative agent of dental plagues and scarlet fever and has been implicated in human infections like pharyngitis, scarlet fever and pneumonia (Adebayo- Tayo *et al.,* 2009).

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