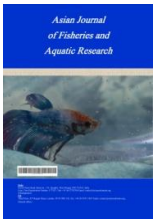

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Microbial Communities of Culture Water and African Catfish Reared in Different Aquaculture Systems in Nigeria Analyzed Using Culture Dependent Techniques

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Authors' contributions

This work was carried out in collaboration between both authors. Author DEU designed the work, directed the protocols and supervised the carrying out of the work. Author MTO carried out the day to day running of the work. Author DEU interpreted the results, wrote the manuscripts. Both authors read and approved the final manuscript.

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ABSTRACT

The microbial communities of culture water and catfish *C. gariepinus* from three replicates of earthen, concrete and tarpaulin ponds in Nigeria were analyzed. Waters was collected from 25 cm below pond water surface per culture system. Three catfish per replicate system were also collected and analyzed in the lab. Catfish gut, skin and gills were analyzed. Earthen ponds had significantly more diverse microbial community and coliform forming units (CFU/ml) 2.43 $x10^{-4}$ CFU/ml than the rest systems. Earthen ponds had consortium of *Klebsiella pneumonia*, *S. aureus*

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and *Salmonella enteritidis* and *E. coli*, which was more diverse than all other aquaculture systems. Microbiota of tarpaulin ponds was $2.10x10^{-4}$ /ml CFU and this was significantly ($P<0.05$) higher than concrete ponds ($1.50x10^{-4}$ CFU/ml). Tarpaulin ponds had *K. pneumoniae* and *E. coli*, while concrete pond had *S. aureus* and *S. enteritidis*. Biofilm formation could have lead to colonization of the fish body part. The skin and gills had similar microbiota as the culture water compared to the gut. The gut microbial communities were not synonymous with the culture water.

Keywords: African catfish; microbiota; aquaculture systems; fish gut microbiota; fish culture water.

1. INTRODUCTION

The culture of African catfish *Clarias gariepinus* is booming in Sub Saharan Africa [1,2]. Aquaculture can enhance food security and alleviate shortfall from capture fisheries [3]. In Sub Saharan Africa the government is encouraging aquaculture to sustain food security and provide protein to the teeming population [4]. Aquaculture has the capacity to surpass the capture fisheries by 2030 especially in Sub Saharan Africa where fish importation is very high [4]. However the boom in culturing of African catfish has lead to use of different culturing systems and unprofessional practices resulting in outbreak of parasitic infections in farms and hatcheries [5]. The culture systems water and environments harbor different microbial communities which affects the microbiota of the fish and gastro intestinal tract (GIT). Fish are associated with the microorganisms in their environment [6,7,8]. The health status of cultured fish and its safety for human consumption is culture system dependent. Cultured fish harbor different microbes emanating from their culture systems. Microbial load of farmed fish has been noted to be determined by the quality of the water used in their culture system [9,10]. Moreover microbiota like *Pseudomonas spp* found in the gut of Atlantic salmon originated from water [11]. Similarly, abiotic factors like dietary input, the surrounding habitat, season and developmental stage had been noted to be influencing fish gut microbiota [12,13,14]. Conversely some workers have stated that gut microbiota of fish changes as soon as fish starts exogenous feeding, such that microbiota would resemble that of feed more than those in their water [15,16,17,18].

Fish foods also contribute in determining the microbial communities of fish gastrointestinal tract. The association that fish have with their environmental microbial communities can be pathogenic, mutualistic or symbiotic. Fish microbiota play key roles in the health, nutrition and identification of where fish was originally cultured or caught before processing. The fish symbiotic gut microbiota are implicated in their nutritional, immune system and metabolic homeostasis [19,20,21]. The fish microbiota communities influence host body mechanisms like larval development, disease resistance and immunity development of the mucosal system and angiogenesis [22,23]. It had been noted also that from early larval stages, the epithelial surfaces of fish is colonized by numerous microorganisms (microbiota) which together relate with their host commensally or mutual manner [24]. Functional analysis of the microbiomes of rainbow trout showed some proof that suggests contributory effects of the microbes to the ingredients dietary metabolism therefore actively influencing the digestive process in the fish [25]. The microbiota in the fish environment can colonize and adhere to the host gastrointestinal tract epithelial tract and are known as autochthonous but when they cannot they are called allochthonous [26,27,28,29,30]. Nevertheless the type and composition of microbial communities is highly influenced by the properties of environment where they are found [31]. Fish is cultured in different rearing systems example recirculation aquaculture systems (RAS), earthen ponds, concrete ponds tarpaulin collapsible tanks and cages. The microbiota of Nile tilapia *Oreochromis niloticus* cultured in recirculation aquaculture system and active suspension tanks were found to be significantly different [32]. The authors did not find any significant difference between microbiota of tilapia from replicates of similar culture system. So far studies investigating microbial communities in fish culture system like RAS [33,34] and the culture fish is yet novel [35]. However it has been noted that the composition of the gastrointestinal microbiome of rainbow trout reared in different aquaculture systems like raceways, earthen ponds and inshore tank systems can be rearing system influenced [14,36,37].

Culture dependent system utilizes biochemical tests of the microbes. Biochemical tests are done

using suspensions of organisms and chemically-defined solutions. The biochemical test utilizes the preformed enzymes of the isolated bacterial cells. In carrying out the biochemical test, cautions should be taken so that results would not be complicated by side effects or by the multiple reactions that could occur in cultures growing in a nutrient media that contained test substrate. Among the biochemical tests to be utilized in this research are oxidase test, urease test, catalase test, coagulase test, indole test, nitrase reductase test, citrate test, manitol test, methyl red test, Voges Proskauer test, H2S test and sugar fermentation test. This research seeks to find the microbial communities composition of culture systems like earthen pond, concrete ponds and tarpaulin collapsible tanks used in rearing African catfish *Clarias gariepinus*. This research also seeks to find composition of the gut microbiota of African catfish cultured in these different culture systems and would analyze if gut microbiota are synonymous to that of the culture water environment.

2. MATERIALS AND METHODS

2.1 Geography of Study Area

This study was carried out from the months of March to end of May 2017. The study was carried out in Enugu, the colonial capital of Eastern Nigeria. It has latitude of 6°27'30.12"N and a longitude of 7°32'47"E (Fig. 1). It was founded by the British in 1900. It has about 10 major lakes and two major rivers Ekulu River and Iyaba River. There are huge deposits of coal, iron ore and gas in Enugu. Presently Enugu is the largest town in Eastern Nigeria and one of the fastest growing state capitals in the country.

2.2 Experimental Fish and Farm

African catfish *Clarias gariepinus* ranging from 30.0cm to 43.2cm in length and 290.2g to 468g in weight used for this work were obtained from the concrete tank fish farms of department of Biological Science Godfrey Okoye University Thinkers Corner Emene Enugu Nigeria. The University owns concrete tanks of 32 feet x 18 feet, dept were 10 feet. The pond was not surface tank but dug into the ground. The pond was 2/3 filled with water. The catfish of similar sizes as stated above were obtained from earthen pond and tarpaulin collapsible pond of a commercial fish farm located at Awkunanaw Enugu.

2.3 Culture Water

The pond water was collected with 250 ml amber colored bottle. The bottle was lowered to about 25 cm below water surface and water was collected without air bubbles. The water was used in analyzing for microbial load of culture water and for physio-chemical parameters. The water parameters analyzed were, pH, dissolved oxygen, total gas pressure, ammonia, temperature, conductivity, turbidity, nitrate, total dissolved solid, ammonia and alkalinity and temperature. Earthen pond water pH was 6.9 ± 0.1, while the concrete pond pH was 6.0 ± 0.3, while the tarpaulin pond pH was 7.0 measured with Combo pH & EC meter; model HI 98129 Hanna Instrument, Arizona USA. Average dissolved oxygen was 7.2 ± 0.2 mg L^{-1} for the earthen ponds, 8.2 ± 0.5 mg L^{-1} for the concrete ponds and 7.86 mg L^{-1} for the tarpaulin ponds, measured with YSI oxygen meter model 550A (YSI Inc. Yellow Springs, Ohio, USA) and total gas pressure was (101.5±1.0% for the earthen ponds, 98.09±1.0% for the concrete ponds and 95.08±0.061.0% for the tarpaulin ponds, measured with P4 Tracker total gas pressure meter (Point Four Systems Inc., Richmond BC, Canada). Ammonia was (0.25±0.07 mg L^{-1} for the earthen ponds, 1.23±0.01 mg L^{-1} for the concrete ponds and 1.85±0.02 mg L^{-1} for the tarpaulin ponds, measured with ammonia test kit (Tetra Merke, Melle, Germany). And alkalinity 1.13±0.01 mmol L^{-1} for the earthen ponds, 0.74± 0.06 mmol L^{-1} for the concrete ponds and 0.82 ± 0.02 mmol L^{-1} for the tarpaulin ponds, measured with test kit Tetra Merke, Melle, Germany. Temperature was averaged 28.01±0.02°C for the earthen ponds, 29.0±0.01°C for concrete ponds and 31.01±0.03°C for the tarpaulin ponds, measured with Celsius mercury in glass thermometer. Nine African catfish were obtained from the ponds (three each from the three replicate ponds.

2.4 Preparation of Agar Media

In carrying out this research nutrient agar, MacConkey agar and eosin methylene blue agar were used. The agar was prepared according to instructions from manufacturer. Approximately 28 g of the different agar were measured into a round bottom flask. The agar was mixed with 1000 ml of distilled water and autoclaved at 121.0°C for 15 minutes. The autoclaving dissolved and gelatinized the agar water mixture. The media was allowed to cool and poured into sterile disposable petri dishes and allowed to solidify.

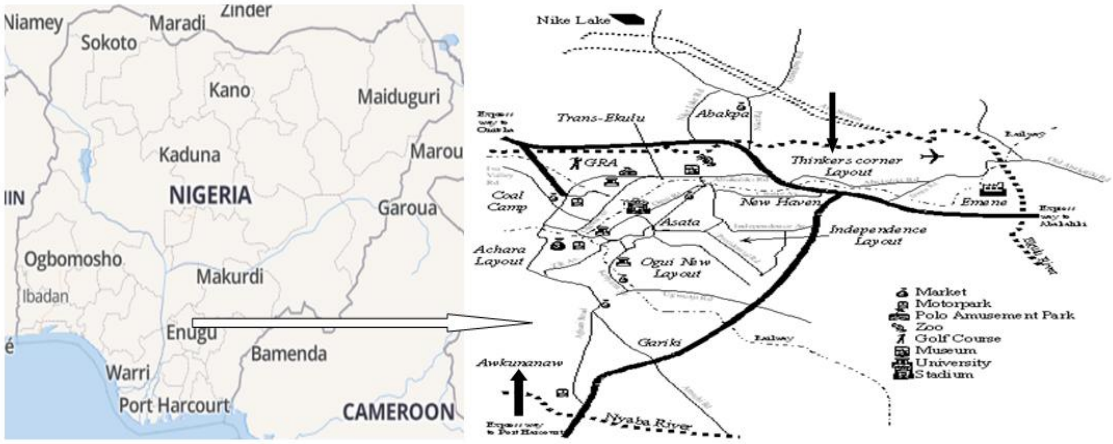

Fig. 1. Map of Nigeria showing Enugu state within Nigeria map with a black diamond. Enugu is the capital of Enugu state in Eastern Nigeria. Black arrows shows the position of the farms where samples were collected (www.igboguide.org) assessed 3.13.2019

2.5 Bacteriological Analysis of the Pond Water

The collected pond water were taken to the lab with 250 ml amber colored bottle and analyzed for bacteria load. Appropriate sample dilution were made (10^{-2} -10^{-4}) with distilled water. Aliquots of 1 ml of serial dilutions were inoculated using pour plate technique on nutrient Agar, MacConkey Agar and Eosin methylene blue Agar. The plates were incubated at 37°C for 24 – 48 hrs. The plates were prepared from water samples from three replicates of each experimental farm.

2.6 Processing of Sample Fishes

Nine adult African catfish of average weight 589.7±10.21 g were collected from each replicate of the earthen, concrete and tarpaulin ponds. Fish were killed with a gentle blow on their head, dissected and the gut system was exposed. The gut was divided into the foregut, the midgut and the hindgut systems. The foregut started from the esophagus to the beginning of small intestine after the duodenum. The midgut was the whole small intestine. The hind gut was taken from the beginning to the end of the large intestine. The gut sections were cut with a surgical blade and place in a 10 ml distilled water. About 5 cm of the skin of the catfish was also cut and placed 10 ml distilled water. The fish body parts were macerated with a sterile pestle and mortar. Aliquot solutions of macerated samples (2 ml) were pipette into 18 ml of distilled water giving 1:10 ml stock sample solution dilution. The stock solution was serially diluted up tom 10^{-5} as described by [38]. Spread plate method was used in inoculating 0.1 ml of the dilution on nutrient agar in duplicate plates using 10^{-2} and 10^{-4}. The inoculated plates were then incubated at 37°C for 24 hrs. Sub culturing was done to obtain pure cultures, and these were inoculated on nutrient agar plates and incubated at 37°C for 24 hrs. The plates were examined after 24 hrs and the number of colony forming units (CFU) on the plates were counted and recoded. Similar procedures was used for both the water analysis, the fore gut, midgut and hindgut of the catfish and skin samples of the fish. The isolates were subjected to morphological and biochemical characterization.

2.7 Gram Staining Technique and Microscopy

The Gram staining technique was used as the staining reaction to identify the different bacteria species by their Gram reaction (Gram +ve or Gram –ve) and their morphology. Gram staining of the isolates was done according to methods stated in the Bergey's manual of determinative bacteriology [39]. The morphological chara-cteristics that were examined includes, colour, edge, elevation, shape and arrangement of microorganisms and motility. A loop ful of the bacterial colonies isolated was emulsified in sterile distilled water and a thin preparation was made on a glass slide. The smear was air-dried completely and rapidly passed through the flame of a spirit lamp and allowed to cool. The fixed smear was flooded with crystal violet stain for 60

seconds, after which it was washed off with sterile water and air dried. Lugol's iodine was applied on the smear and allowed for 60 seconds and later washed off with sterile water. The smear was decolorized with ethanol for 30 seconds and immediately washed off with sterile water. Safranin was used to flood the smear for about 2 minutes and later washed off with sterile water. The back of the slide was wiped clean and placed in a drying rack for the stained smear to air-dry. The examination of microorganisms under slide was made in oil immersion after Gram staining. All Gram stained smears of different colonies from different cultures were examined using oil immersion objectives (x100) photoscope microscope. This was in order to check the bacteria staining reaction and morphology of the bacteria species [40,41].

2.8 Biochemical Tests

Biochemical tests carried out were as follows; oxidase test [42] the colour change was observed after the rubbing. The result was judged oxidase +ve when the colour changes to dark purple within 5 to 10 seconds. Conversely the result was considered oxidase –ve if the colour does not change or it takes longer than 2 minutes [42]. Urease test, Results were judged based on development of a bright pink colour indicating a +ve reaction. The reverse was –ve [38]. Catalase test, Results is +ve catalase test if there is active bubbling in the test tube and –ve catalase test if there are no bubbles in the test tubes [43]. Coagulase test was carried after [43]. Clumping was a test indicator. The observed results for clumping within 10 seconds was coagulase +ve while, no clumping within 10 seconds were noted recorded as coagulase –ve [43]. Indole test was carried out after [38]. A change in colour of the system was used as indication of +ve or –ve Indole test. A red color appearing in the surface layer of the tryptone water- Kovac's reagent mixture identified Indole +ve. The reverse was Indole –ve respectively [38]. H2S (sulphate reductase test) was carried out after [38]. The result was judged +ve based on observation of black colouration at the point of stab. The reverse case was –ve [38]. Citrate test was carried out after [38]. The results were based on observation of colour change. Colour change from pale green to blue indicated a +ve result. Citrate +ve: growth was visible on the slant surface and the medium colour was intense Prussian blue. The result was Citrate –ve if mere trace or no growth was visible. Analysis of Mannitol test was sugar test [38]. Mannitol is a sugar that some bacteria can use because of an enzyme that breaks down the compound. The test is judged +ve if the colour turn from usual red to yellow. The reverse is the –ve mannitol [38]. Methylred test was also carried out after [38]. Result was judged +ve at the formation of a red colour. The result was considered –ve reaction if there were yellow colour instead [38]. Voges-Proskauer test was also carried out after [38]. The result was judged +ve at the appearance of a pink colour after 24hrs indicated a +ve result [38].

2.9 Statistical Analysis

The results were analyzed using one way analysis of variance (ANOVA). Fishers least significant difference (LSD) 0.05 was used in separating possible differences of treatment means. SPSS version 14.0 statistical package was used for analyses.

3. RESULTS

3.1 Microbial Communities of Culture Systems

The different aquaculture systems harbored different microbial communities which are also reflected on the fish. Culture water obtained from the earthen ponds harbored bacteria communities between 2.04 $x10^{-4}$ CFU/ml and 2.87 $x10^{-4}$ CFU/ml (Table 1). The average CFU/ml of the microbiota from the earthen ponds water was 2.43 $x10^{-4}$ CFU /ml (Table 1). The water sample from concrete pond fish culture system had microbiota of between $0.99x10^{-4}$ CFU/ml to $1.98x10^{-4}$ CFU/ml. The average CFU/ml of the microbiota from the concrete pond water was $1.50x10^{-4}$ CFU which was significantly ($P<0.05$) lower than that of the earthen pond (Table 2). Conversely, the culture water from tarpaulin ponds had microbial communities of between $1.90x10^{-4}$ CFU/ml to 2.25 $x10^{-4}$ CFU/ml. The average CFU/ml of the microbiota from the tarpaulin ponds was $2.10x10^{-4}$ CFU/ml and this was higher than concrete tanks. There was significant differences ($P>0.05$) between the microbiota CFU/ml of tarpaulin ponds and that of concrete ponds ($P<0.05$) (Table 2). The microbiotas of the fish were similar to that of their aquaculture systems. The fish microbiota according to culture systems are tabulated from Tables 3 - 4. Results of morphological and biochemical test analysis of the culture water from earthen ponds, concrete ponds and tarpaulin ponds are recorded in Tables 4,6. The

results showed that microbial communities on the earthen ponds comprises consortium of Gram--ve rod *Klebsiella pneumoniae, Gram +ve* cocci *S. aureus,* Gram -ve rod *Salmonella enteritidis and Escherichia coli.* The water from concrete ponds showed that the microbiome was a combination of Gram +ve cocci *Staphylococcus aureus* and Gram -ve rod *Salmonella enteriditis.* There were equal compositions of the two microbiota organisms. The water from tarpaulin ponds was analyzed and noted to contain a combination of Gram -ve rod *Klebsiella pneumoniae* and Gram -ve rod E. coli. The biochemical and Gram staining analysis of the water showed that the microbiota of the water from earthen ponds harbored different microorganisms (Tables, 5, 6,7). The earthen ponds culture water was dominated by a consortium of bacteria. Dominant among the bacteria were Gram –ve rod *Klebsiella pneumoniae* which were identified in the catfish specimen. Also isolated from the earthen pond culture system water was a Gram +ve cocci species *Staphylococcus aureus*. Other microbes isolated were Gram -ve rods namely *Salmonella enteriditis* and the fecal bacteria *Escherichia coli.* The culture system enhanced a consortium of these microbial communities (Tables 4,6).

3.2 Microbial Communities of Fish Body Parts

The analysis of the microbiota CFU/ml from skin, gills, foregut, midgut and hindgut of catfish cultured in earthen ponds showed variation such that, skin had between 1.00 $x10^{-4}$ CFU/ml to 1.20 $x10^{-4}$ CFU/ml while the gills harbored from 0.80 $x10^{-4}$ CFU/ml to 1.00 $x10^{-4}$ CFU/ml. The foregut had between 1.30$x10^{-4}$ CFU/ml to 1.20 $x10^{-4}$ CFU/ml while the midgut harbored 1.00$x10^{-4}$ CFU/ml to 1.80 $x10^{-4}$ CFU/ml. The hindgut harbored between 1.90$x10^{-4}$ CFU/ml to 2.45 x 10^{-4} CFU/ml (Table 3). The somatic microbiota of catfish cultured in concrete ponds was lesser than the earthen ponds. The microbiota CFU/ml from skin, ranged between 0.20 $x10^{-4}$ CFU/ml to 0.80 $x10^{-4}$ CFU/ml while the gill harbored between 1.00 $x10^{-4}$ CFU/ml to 1.40 $x10^{-4}$ CFU/ml. The average quantity of microbiota in the gill was 1.20 $x10^{-4}$ CFU/ml (Table 3). The catfish cultured in Tarpaulin collapsible ponds had skin microbiota of between 0.55$x10^{-4}$ CFU/ml to 0.80 $x10^{-4}$ CFU/ml. The average

Table 1. Results of bacteria colonies of pond water from earthen ponds, concrete ponds and tarpaulin pond after 48 hrs incubation

A. systems	Inoculums Volume	Dilution Factor	No colonies	Total no of organism CFU/ml
Earthen	1	0.1	24	2.04 $x10^{-4}$ CFU
ponds	1	0.3	32	2.32 $x10^{-4}$ CFU
	1	0.5	39	2.87 $x10^{-4}$ CFU
				2.43 $x10^{-4}$ CFU
Concrete	1	0.1	12	0.99$x10^{-4}$ CFU
ponds	1	0.3	15	1.60$x10^{-4}$ CFU
	1	0.5	20	1.98$x10^{-4}$ CFU
				1.50$x10^{-4}$ CFU
Tarpaulin	1	0.1	22	1.90$x10^{-4}$ CFU
ponds	1	0.3	26	2.15$x10^{-4}$ CFU
	1	0.5	28	2.25 $x10^{-4}$ CFU
				2.10$x10^{-4}$ CFU

Where A. systems is aquaculture rearing system, CFU is colony forming units

Table 2. Bacteria load of water from different aquaculture systems in Nigeria used in lturing African catfish *C. gariepinus* and different body parts of the cultured fish analysed viz:viz: skin, gills, foregut, midgut and the hindgut

Ponds	Culture water	Skin	Gills	Foregut	Midgut	Hindgut
Earthen	2.4$x10^{-4a}$	1.0$x10^{-4a}$	0.9$x10^{-4c}$	1.6$x10^{-4b}$	1.4$x10^{-4a}$	2.2$x10^{-4a}$
Concrete	1.5$x10^{-4c}$	0.5$x10^{-4b}$	1.2$x10^{-4b}$	2.1$x10^{-4a}$	1.1$x10^{-4b}$	2.2$x10^{-4a}$
Tarpaulin	2.1$x10^{-4v}$	0.7$x10^{-4b}$	2.0$x10^{-4a}$	1.1$x10^{-4c}$	0.8$x10^{-4c}$	0.6$x10^{-4b}$

Means in the same column not followed by the same superscript are significantly different (P<0.05)

Table 3

Results of bacteria colonies of skin, gills, foregut and hind gut Of African catfish cultured in earthen ponds, Tarpaulin ponds and concrete ponds in Nigeria

					total number of organisms		
	Variables	Innoculum vol	Dilution factor	no colonies	earthen pond	Tarpaulin pond	Concrete pond
	Skin	1	0.1	3	1.00×10^{-4} CFU	0.55×10^{-4} CFU	0.20×10^{-4} CFU
		1	0.3	4	1.01×10^{-4} CFU	0.75×10^{-4} CFU	0.50×10^{-4} CFU
		1	0.5	6	1.20×10^{-4} CFU	0.80×10^{-4} CFU	0.80×10^{-4} CFU
					1.00×10^{-4} CFU	0.7×10^{-4} CFU	0.50×10^{-4} CFU
	Gills	1	0.1	1	0.80×10^{-4} CFU	1.50×10^{-4} CFU	1.00×10^{-4} CFU
		1	0.3	3	0.90×10^{-4} CFU	2.20×10^{-4} CFU	1.20×10^{-4} CFU
		1	0.5	3	1.00×10^{-4} CFU	2.30×10^{-4} CFU	1.40×10^{-4} CFU
					0.90×10^{-4} CFU	2.00×10^{-4} CFU	1.20×10^{-4} CFU
	Foregut	1	0.1	2	1.30×10^{-4} CFU	1.00×10^{-4} CFU	2.00×10^{-4} CFU
		1	0.3	4	2.30×10^{-4} CFU	1.30×10^{-4} CFU	2.30×10^{-4} CFU
		1	0.5	2	1.20×10^{-4} CFU	1.00×10^{-4} CFU	2.00×10^{-4} CFU
					1.60×10^{-4} CFU	1.10×10^{-4} CFU	2.10×10^{-4} CFU
	Midgut	1	0.1	2	1.00×10^{-4} CFU	0.40×10^{-4} CFU	0.30×10^{-4} CFU
		1	0.3	3	1.4×10^{-4} CFU	0.90×10^{-4} CFU	1.00×10^{-4} CFU
		1	0.5	4	1.80×10^{-4} CFU	1.10×10^{-4} CFU	2.00×10^{-4} CFU
					1.40×10^{-4} CFU	0.80×10^{-4} CFU	1.10×10^{-4} CFU
	Hindgut	1	0.1	3	1.90×10^{-4} CFU	0.10×10^{-4} CFU	1.10×10^{-4} CFU
		1	0.3	5	2.25×10^{-4} CFU	0.50×10^{-4} CFU	1.10×10^{-4} CFU
		1	0.5	7	2.45×10^{-4} CFU	1.20×10^{-4} CFU	1.40×10^{-4} CFU
					2.20×10^{-4} CFU	0.60×10^{-4} CFU	1.20×10^{-4} CFU

Where CFU is colony forming unit

Table 4. Results of gram stain of streaked colonies of culture water from earthen ponds, concrete ponds and tarpaulin collapsible ponds after 48 h of incubation and plausible organisms

System	Dilution factor	Colour	Gram stain colonies	Cell type	Shape	Cell Arrangement	Probable org
Earthen Ponds	0.1	Cream	-ve	Rod	irregular	Single	*Klebsiella pneumoniae*
	0.1	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.3	Brown	-ve	Rod	Straight	Single	*Salmonella enteritidis*
	0.5	Light red	-ve	Rod	Straight	Single	*E. coli*
Concrete	0.1	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
ponds	0.3	Brown	-ve	Rod	Straight	Single	*S.enteritidis*
	0.5	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.5	Brown	-ve	Rod	Straight	Single	*S.enteritidis*
Tarpaulin	0.1	Cream	-ve	Rod	irregular	Single	*K. pneumoniae*
ponds	0.3	Light red	-ve	Rod	Straight	Single	*E. coli*
	0.5	Light red	-ve	Rod	Straight	Single	*E. coli*
	0.5	Cream	-ve	Rod	irregular	Single	*K. pneumoniae*

skin microbiota of the catfish was 0.7 $x10^{-4}$ CFU/ml (Table 3) which was lower than that of catfish cultured in earthen ponds. Conversely the gills microbiomes varied from 1.50 $x10^{-4}$ CFU/ml to 2.30 x10-4 CFU/ml with average value of 2.00 $x10^{-4}$ CFU/ml. The gut microbiomes varied as follows, foregut 1.00 $x10^{-4}$ CFU/ml to 1.30x 10^{-4} CFU/ml, midgut, $0.40x10^{-4}$ CFU/ml to 1.10 $x10^{-4}$ CFU/ml and hindgut $0.50x10^{-4}$ CFU/ml to 1.20 $x10^{-4}$ CFU/ml (Table 3). The biochemical analysis of microbiota found in skin, gills, foregut, midgut and hindgut of catfish cultured in earthen ponds showed some resemblance to that of the culture water. The skin of the catfish was noted

to harbor consortium of Gram -ve rod *Klebsiella pneumoniae* and Gram +ve cocci *Staphylococcus aureus.* The catfish gill harbored a consortium of Gram -ve rod *Klebsiella pneumoniae,* Gram +ve rod *Bacillus subtilis* and Gram -ve rod *Proteus mirabilis.* The biochemical analysis of microbial communities of catfish foregut showed that it comprised of Gram +ve rod *B. subtilis* and Gram +ve cocci *Staphylococcus aureus*. The midgut of the earthen pond catfish comprised a consortium of Gram -ve rod *Klebsiella pneumoniae* with Gram +ve cocci *Staphylococcus aureus* and the Gram -ve rod *Pseudomonas aeruginosa*. The microbiotas of the hindgut of the catfish cultured in earthen ponds comprise of Gram -ve rods *Klebsiella pneumoniae* and *Escherichia coli* (Table 5).

3.3 Relationship between Microbiota of Culture System and Fish Body Parts

The gut microbiota of the catfish cultured in concrete ponds resembled that of the culture water (Tables 7, 8). The skin of the catfish cultured in concrete ponds had microbiota comprising of Gram +ve rod *B. subtilis,* Gram -ve cocci *S. aureus* and Gram +ve cocci *Streptococus pneumoniae.* There was more *B.subtilis* CFU/ml in the community than the *S.pneumoniae*. The gills of the catfish cultured in concrete ponds had microbiota comprising of Gram +ve coccis, *S. aureus* and *S. pneumoniae.* The gut microbial communities of the catfish cultured in concrete ponds were similar to that of culture water. The foregut of the catfish comprised of Gram -ve rod *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The midgut of the catfish cultured in the concrete ponds comprised of a consortium of Gram +ve rod *B. subtilis,* Gram -ve rod *Proteus mirabilis* and Gram -ve rod *E. coli.* The hind gut of the catfish was comprised of a combination of two Gram -ve rods *Klebsiella pneumoniae* and *E. coli.*

The catfish cultured in tarpaulin ponds showed microbiotas that are similar to the microbial communities of the culture waters (Table 9). The skin of the catfish has microbial communities comprising of Gram -ve rod *Klebsiella pneumonia* and Gram +ve cocci *S. aureus*. The microbiota of the catfish gill cultured in tarpaulin ponds also resembled the microbial communities of the culture water. The gill microbiota comprised a consortium of Gram -ve rod *K. pnuemoniae*, Gram -ve rod *Salmonella enteritidis* and *B. subtilis*. The catfish gut microbial communities was however similar to the culture water as well. The foregut microbiota was made up of consortium of *K. pneumonia, S. aureus* and *E. coli.* (Tables 9 and 8). Similarly, the midgut of the catfish harbored consortium of *K.pneumoniae*, *E. coli* and *B. subtilis*. The microbiota of the hindgut was consortium of *K. pneumoniae*, *S. aureus* and *E. coli* (Tables 9).

4. DISCUSSION

Fish lives in water and from the egg to adults stages of life that fish lives in water and can be colonized by aquatic bacteria [44]. In a previous research [45] noted that the composition of microbial communities of the gills and gut of *Liopropoma santi* were similar to the bacterial community of their aqueous environment. Similarly, it was noted that the dominant *Pseudomonas spp* of the bacterial flora of yolk-sack larvae of milkfish, *Chanos chanos* (Forsskal), resembled that of the rearing water [46]. Consequently it had been noted that fish acquire bacteria in their gut from their aquatic ecosystem [47] and from drinking rearing water to control osmoregulation [13]. The acquired microbes' communities significantly influence fish health, various host functions including immunity development, disease resistance, digestion, and nutrition [48]. In this research the culture systems seem to have provided enabling environment for the proliferation of the bacterial communities. The earthen ponds had more bacteria counts than the rest of the aquaculture systems. The earthen ponds in this research had consortium of *Klebsiella pneumoniae*, *S. aureus*, *Salmonella enteritidis* and *E. coli*, which formed bases for the complex microbiota of catfish cultured in the system. Consequently the skin of the catfish cultured in earthen ponds had more microbiota than catfish from the rest of the culture systems. Similar scenario were noted for both concrete and tarpaulin ponds. This seems to suggest that culture system influences the microbial communities of fish.

The results of gut microbiota, organ and culture water microbiota suggests that catfish obtain microbes from the aquaculture systems water. This is in line with previous reports that fish microbial communities originates from the culture water [8,49,50,51]. The results of the microbial communities from the sample water of the culture system and the fish inhabiting them shows

Table 5. Results of Gram staining of streaked colonies extracted from skin, gills, foregut, midgut and the hindgut of African catfish Clarias gariepinus cultured in earthen ponds, concrete ponds and tarpaulin collapsible ponds in Nigeria

	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H2S	Nitrase reductase	Methyl red	Voges Proskauer	Probable org
Skin	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*S. aureus*
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*S. aureus*
	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
Gills	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
	Positive	variable	negative	Positive		negative	Positive	N/A	Positive	Negative	Positive	*Bacillus subtilis*
	Positive	variable	negative	Positive		negative	Positive	N/A	Positive	Negative	Positive	*Bacillus subtilis*
	Positive	negative	negative	Positive		positive	negative	Positive	Positive	Positive	negative	*Proteus mirabilis*
Foregut	Positive	variable	negative	Positive	N/A	negative	Positive	N/A	Positive	Negative	Positive	*Bacillus subtilislis*
	Positive	variable	negative	Positive	N/A	negative	Positive	N/A	Positive	Negative	Positive	*B. subtilis*
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*S. aureus*
	Positive	negative	Positive	negative	N/A	negative	Positive	negative	Positive	Positive	negative	*Escherichia coli*
Midgut	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*K. pneumoniae*
	Positive	variable	negative	Positive	N/A	negative	Positive	N/A	Positive	Negative	Positive	*Bacillus* spp
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*S. aureus*
	Positive	Positive	negative	Positive	negative	negative	Positive	negative	Positive	negative	negative	*Pseudomonas* spp
Hindgut	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
	Positive	negative	negative	Positive	N/A	positive	Positive	negative	Positive	Positive	Positive	*Klebsiella pneumoniae*
	Positive	negative	Positive	negative	N/A	negative	Positive	negative	Positive	Positive	negative	*Escherichia coli*
	Positive	negative	Positive	negative	N/A	negative	Positive	negative	Positive	Positive	negative	*Escherichia coli*
F2	Positive	negative	negative	negative	N/A	negative	positive	Positive	Positive	Positive	negative	*Salmonella* spp
	Positive	Positive	negative	Positive	negative	negative	Positive	negative	Positive	negative	negative	*Pseudomonas* spp
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*Staphylococcus* spp
	Positive	negative	negative	positive	Positive	Positive	Positive	negative	Positive	Posssitive	Positive	*Staphylococcus* spp
	Positive	negative	Positive	negative	N/A	negative	Positive	negative	Positive	Positive	negative	*Escherichia coli*

Table 6. Results of biochemical test analysis of culture water from earthen ponds, concrete ponds and tarpaulin collapsible ponds used in culturing African catfish *Clarias gariepinus* in different farms in Nigeria

System	catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H2S	N. reductase	Methyl red	VP	Organism
Earthen	+ve	-ve	-ve	+ve	N/A	variable	+ve	+ve	+ve	+ve	-ve	*Klebsiela pneumoniae*
ponds	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+Ve	*Staphylococcus aureus*
	+ve	-ve	-ve	-ve	N/A	-ve	+ve	+ve	+ve	+ve	-ve	*Salmonella enteritidis*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
Concrete	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+Ve	*Staphylococcus aureus*
ponds	+ve	-ve	-ve	-ve	N/A	-ve	+ve	+ve	+ve	+ve	-ve	*Salmonella enteritidis*
	+ve	-ve	-ve	-ve	N/A	-ve	+ve	+ve	+ve	+ve	-ve	*Salmonella enteritidis*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	--ve	+ve	+ve	+Ve	*Staphylococcus aureus*
Tarpaulin	+ve	-ve	-ve	+ve	N/A	Variable	+ve	+ve	+ve	+ve	-ve	*Klebsiela pneumoniae*
ponds	+ve	-ve	-ve	+ve	N/A	Variable	+ve	+ve	+ve	+ve	-ve	*Klebsiela pneumoniae*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*

Table 7. Results of biochemical tests of streaked colonies of bacteria extracted from skin, gills, foregut, midgut and the hindgut of African catfish Clarias gariepinus cultured in earthen ponds, ponds in Nigeria

	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H2S	Nitrase reductas	Methyl red	Voges Proskauer	Probable or
Skin	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
Gills	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	variable	-ve	+ve		-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
	+ve	variable	-ve	+ve		-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
	+ve	-ve	-ve	+ve		+ve	-ve	+ve	+ve	+ve	-ve	*Proteus mirabilis*
Foregut	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilislis*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*B. subtilis*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
Midgut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus* spp
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas* spp
Hindgut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*

Where +ve =positive, -ve = negative, N/A =not applicable

Table 8. Results of biochemical tests of streaked colonies of bacteria extracted from skin, gill, foregut, midgut and hindgut of African catfish *Clarias gariepinus* cultured in concrete ponds in Nigeria

	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H2S	Nitrase reductase	Methyl red	Voges Proskauer	Probable org
Skin	+ve	-ve	-ve	+ve	N/A	-ve	-ve	N/A	+ve	N/A	-ve	*Streptococcus pyogenes*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
Gills	+ve	-ve	-ve	+ve	N/A	-ve	-ve	N/A	+ve	N/A	-ve	*Streptococcus pyogenes*
	+ve	-ve	-ve	+ve	N/A	-ve	-ve	N/A	+ve	N/A	-ve	*Streptococcus pyogenes*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
Foregut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas aeruginosa*
	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas aeruginosa*
Midgut	+ve	variable	-ve	+ve		-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*B. subtilis*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	-ve	+ve		+ve	-ve	+ve	+ve	+ve	-ve	*Proteus mirabilis*
Hindgut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*

Where +ve=positive, -ve =negative and N/A =not applicable

Table 9. Results of biochemical tests of streaked colonies of bateria obtained from skin, gills, foregut, midgut and hindgut of African catfish *C. gariepinus cultured* in tarpaulin collapsible ponds in Nigeria

	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H2S	Nitrase reductase	Methyl red	Voges proskauer	Probable org
Skin	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
Gills	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	variable	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*Bacillus subtilis*
	+ve	-ve	-ve	+ve	variable	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	-ve	-ve	N/A	+ve	+ve	+ve	+ve	+ve	-ve	*Salmonella enteritidis*
Foregut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	*S.aureus*
Midgut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*K. pneumoniae*
	+ve	varible	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*B. subtilis*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	-ve	+ve	N/A	-ve	+ve	N/A	+ve	-ve	+ve	*B. subtilis*
Hindgut	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	N/A	+ve	+ve	-ve	+ve	+ve	+ve	*Klebsiella pneumoniae*
	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*S. aureus*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
	+ve	-ve	+ve	-ve	N/A	-ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*

Where +ve=positive, -ve =negative and N/A =not applicable

synchronization. The skin and the gills are in more contact with the culture system water than the gut. Therefore the synchronization and simulation of microbiota of the water, skin and gills could be as results of the contact with the culture water. The fish skin microbiota has been used in showing specific relationship to fish source of origin [52,53] and where fish was cultured prior to processing [54]. The gills of the catfish cultured in earthen pond and tarpaulin ponds have more microbial communities and isolated organisms than the concrete. The consortia of microbes like *B. subtilis, Salmonella enteritidis* and *K. pneumoniae* and *E. coli* could lead to biofilms which may easily form in the earthen ponds and tarpaulin ponds than the concrete ponds, probably due to the cement chemicals. *P. aeruginosa*, is known to be an important pathogen plus avid biofilm former, similarly *Bacillus spp* and *Salmonella spp* [55,56,57]. This could reflect the organisms in the culture system. These highlight the importance of studying microbial communities of the fish in relation to the aquaculture system. Klausen et al., [58] stated that the culture systems used in the culturing of Nile tilapia influenced the proportion of bacterial genera isolated and the bacteria diversity. The authors narrated that the *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Mycobacterium* spp isolated were similar to the culture water.

The differences in the skin and gill microbiota compared to the gut system could be based on contact and feeding. We noted that the microbial communities of the gut are not completely similar to the culture water. This suggests that the gut microbial communities could as well be as a result of the feed. According to literature diets exert much influence in determining complexities of the gut microbial community starting from first feeding larval stages and its diversities [32,59,60, 61,62]. The consortia of microbes like *B.subtilis, Salmonella enteritidis* and *K.pneumoniae* and *E. coli* could have lead to biofilms which were noticeable and could easily form in the earthen ponds and tarpaulin ponds than the concrete ponds due to the cement chemicals. *B. subtilis* is known to form robust biofilm which can disintegrate within 6-8 days [63,64]. Similarly, the biofilm formation of *K.pneumoniae*, *E. coli* and *S. enteritidis* could have been responsible for the colonization of the skins and gills of the catfish. It has been previously noted that bacteria's ability to form biofilm enhances bacterial pathogens to colonize hosts niches and to persist [65]. *P. aeruginosa*, also is known to be an important pathogen plus avid biofilm former [57], similarly *Bacillus spp* and *Salmonella spp* [58,66] also uses several attachment organelles to irreversibly adhere to a surface of hosts. This could be the bases for the synchronization of microbial communities in the culture water to the cultured catfish skin and gills.

5. CONCLUSION

Culture systems affected the microbial communities of the African catfish. The earthen pond had more diverse microbial communities followed by the tarpaulin pond and then finally the concrete ponds. The formation of biofilms seems to be instrumental to the similarities of the microbiota of the catfish and bacteria communities of the water in culture system. The similarities in the catfish and culture water microbial communities were noted more in the skin and gill of the catfish than the gut system. It seems that gut microbial communities could have been influenced more by the feeding since diverse organisms isolated from the gut were not present in the aquaculture system culture water.

COMPETING INTERESTS

AuthorS have declared that no competing interests exist.

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