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Bacteria Associated with Some Freshwater Fishes in Dangana Lake Lapai, Nigeria.

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Abstract
The prevalence of bacteria associated with some freshwater fishes in Dangana Lake were studied. A total of 184 fishes belonging to four species were investigated which were *Clarias gariepinus, Tilapia zilli, Oreochromis nilocticus*, and *Leptocypris nilocticus*. Pour plate and surface streaking techniques were used. identification and characterization of various isolates were based on gram-staining technique and some biochemical tests. From the sampled fishes the total mean colony count of dilution factors (10^6 10^7 10^8) for *Clarias gariepinus* body were 49.00±3.58×, 48.20±4.15, and 47.00±1.58cfu/ml, *Clarias gariepinus* gill 51.00±4.67, 50.20±3.68, 44.20±1.68cfu/ml and in *Tilapia zilli* body 49.20±5.08 ,42.60±2.36,44.40±2.04cfu/ml and in the gill show a mean population of 46.00±6.70 43.20±3.733 48.20±2.54 cfu/ml. *Oreochromis nilocticus* body 54.30±6.38, 59.00±3.78, and 39.60±3.18 were the gill show a mean population of 43.00±7.37, 53.60±6.98cfu/ml, and 48.00±.1.15cfu/ml. The means population of *Leptocypris nilocticus* body 56.40±3.23, 47.20±1.77, and 48.00×±3.16 cfu/ml. Total of 6 bacteria species were isolated and identified from the gill and bodies of the fish, they include *Staphylococcus aureus, Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp. *Proteus* sp. and *Micrococcus* sp. The study revealed that aquaculture products are prone to attack by different groups of microorganisms.

Keywords: Lake, Bacteria, Fishes, Sample, Lapai.

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Introduction

Fishes have remarkable impact on the lives of many individual and communities, as a major source of relatively cheap and affordable animal protein (Ekanem *et al.*, 2011; Eyo *et al.*, 2011). The ever-increasing cost of beef makes fishes feasible option in resolving protein shortage. Fish are very rich source of protein and contains lipids, mineral oils, and vitamins, another product of fish aside fish meal is fish oil which contains omega-3-essential fatty acid necessary for the proper functioning of the brain, heart and immune system (Ashade *et al.*, 2013). Fish interact with various level of food chain and influence the structure their habitat, as they are usually restricted to particular mode of life related to their food source and reproductive requirement (Ashade *et al.*, 2013). Since 70% of the earth's surface is covered by water, there are plenty sources to harvest fish from. Fishes are found in different waters. Some fishes are found in fresh water while some are found in salt water, however, the type of microorganism found associated with a particular fish depends on the water habitat

they are found (Eze *et al.*,2011). The role of freshwater fish in transmitting parasites had been known for a long time. Fish parasites and disease remain one of the problems confronting the fishery biologist, as fish may serve as a parentenic intermediate or definitive host of parasites that are harmful to man and animal (Ravinchandran *et al.*,2007).

Fishing is one of the main reasons communities settles around water bodies (Adamu *et al.,* 2018) As these communities settles around the water bodies, they are known to participate in changing the ecology of the water where bacteria that are part of the aquatic biodiversity have received less attention (Adamu *et al.,* 2018). Studies have shown that bacteria do not only exist in the water but can live on/in aquatic biota like the macrophytes and the fishes. Studies have also shown that fish are host to bacteria species as they are the most causative agents of fish diseases (Shinkafi and Ukwaja, 2010,; Anyanwu *et al*., 2015, Olugbojo and Ayoola, 2015; Adamu *et al.*,2018; Adamu *et al.,* 2018). Many fishes have being found to harbour plenty of protozoan, helminthes, nematodes and bacteria which are either ecto or endoparasites. These parasite are known to affect fish health, growth and survival. The effect of parasites on fish include nutrient devaluation, alteration of biology and behaviour, lowering of immune capability, induction to blindness, morbidity, mortality growth, fecundity reduction and mechanical injuries depending on the parasite species and the load (Ekanem *et al.,* 2011; Eyo *et al.,* 2011). Aquaculture products can harbor pathogenic bacteria which are part of the natural microflora of the environment. Human infections caused by pathogen transmitted from fish or the aquatic environment are quite common depending on the season, patients contact with fish and related environment dietary habit and immune system statue of the exposed individual (Acha and Szyfres, 2003). Food contamination caused by bacteria often results in food spoilage causing life threatening health implication like food poisoning (Moshood and Tenghaziyamin, 2012). Prevention thus helps in the preservation of food quality and public health enhancement. Therefore, studying the distribution of bacteria isolates in Dangana lake Lapai Niger state does not only reveal the potential pathogenic bacteria distribution but the possible reduction or improvement of nutritional and healthy nature of the biota as it directly or indirectly affect human.

Materials and Methods

Study Area.

The study was carried out at Dangana lake, Lapai, Niger state, Nigeria. This lake is located within longitude 6°36’29.6′E and latitude 9°02′12.02N with elevation of 159m above the sea level. The vegetation of the area reflects that of Savannah zone, the vegetation are mixed, prominent ones include Malaina (*Gmeilana arborea*) Locust beans (*Parkia biglobosa*) Neem (*Azadirachta indica*) and other sparsely native trees and grasses. The climate presents two distinct seasons, a rainy season between April and October, and a dry season (November-March) completely devoid of rain.

Fish Collection and Identification.

Fish samples were obtained using gill net and cast net during the sampling period of 10months, February to November 2014 with the assistance of hired fishermen in the lake. The fishes collected were identified with the aid of keys provided by Idodo-Umeh (2003) and Olaosebikan and Raji (2004)

Bacteria Sample collection and Examination

Samples were collected immediately on the field using a sterile swab stick. The swab stick was used by swabbing the fins, body and gills of the sampled fishes. Two methods of sampling were employed. The spread plate method adopted by (Cheesbrough, 2006) where sampled was prepared by using spread plate by surface streaking the swab on a solidified prepared nutrient agar. And pour plate method as described in Olayemi *et al*., (1990). Each fresh fish body were swabbed with a

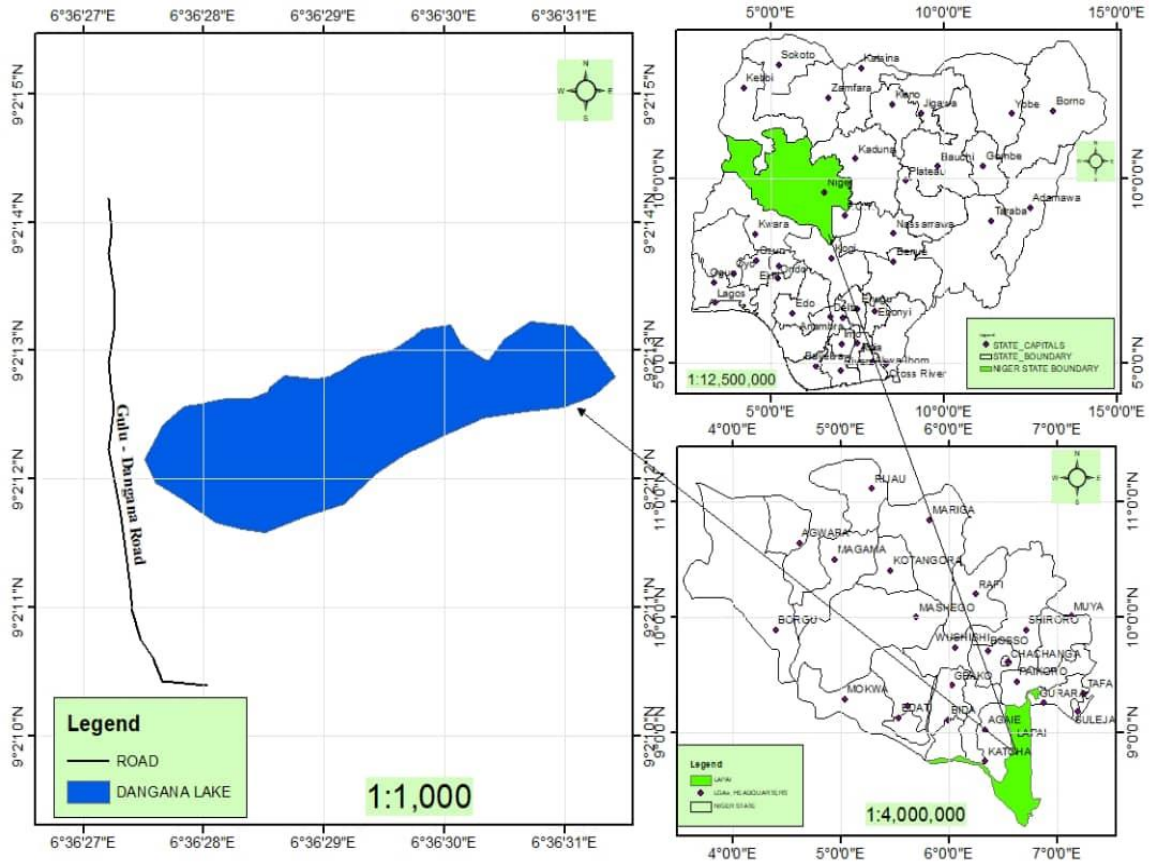

Fig. 1: Geographical location of Dangana Lake Lapai, Niger State.

sterilized swab stick. Thereafter the swab sticks were inserted into test tube containing 9ml of distilled water as a stock, and nine other test tubes also containing 9 ml of distilled water were arranged serially in the test tube rack. 1 ml. of the stock was collected using a pipette to the first test tube and from the first test tube to the second test tube up to the ninth test tube respectively. 10^{-5}, 10^{-6} and 10^{-7} were used as the dilution factor and 1 ml. was taken from each factor into a sterilized Petri dish in duplicate.

Nutrient agar was prepared by dissolving 28.0g in 1litre of distilled water. The dissolved nutrient agar was then autoclaved at 121°C temperature for 15 minutes according to manufacturer's guide (BIOTEC nutrient agar manufactured by BIOTEC laboratory Ltd, Suffolk IP5 3RG United Kingdom). The media was allowed to cool down in sterilized chamber and pour into each Petri dishes containing 1 ml. of the diluents. There after allowed to solidify and incubate at temperature of 37°C for 24 hrs.

Bacteria Colony Count

Bacteria colonies were counted using colony machine (Model R250000614 manufactured by Stuart Scientific Co. Ltd., Great Britain). The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1 ml of original sample, and plating was done in duplicate foe each dilution. An average count was taken to obtain the total count and the results were recorded as colony forming units per millilitre (cfu/ml) of sample (Ibrahim *et al*., 2014)

Identification and Characterization of the Isolates

All isolates were sub-cultured and transferred to a slant media to obtain a pure culture where a gram-staining was conducted to identify the isolates based on the method described by Cheesebrough (2006). Thereafter, the various biochemical tests were conducted for further identification and characterization of the isolates. The cellular morphology of the bacteria isolates was examined by studying their reaction to Gram stain and different biochemical tests such as catalase test, coagulase test, methyl red test, indole test, citrate ultilization and Sugar fermentation test (Cheesebrough, 2006).

Data analysis Calculation of mean colony forming unit per ml (CFU m^{-1}) The mean colony forming unit per ml (Cfu/ml) as $\Sigma f\chi/\Sigma f$, (Sichewo *et al.*, 2013).

Results

Fish Examined The total number of fish examined was 184. There were four species of fish examined in the lake which were *Clarias gariepinus, Tilapia zilli, Oreochromis nilocticus,* and *Leptocypris nilocticus* recording a 44, 44, 37, 37 number of species respectively.

Bacteria colony count

Bacteria colony count of fresh fish sample from the lake revealed mean colony count of *Clarias gariepinus* body is 4.90×10^6, 4.82×10^7, and 4.7×10^8cfu/ml *Clarias gariepinus* gill 5.10×10^6, 5.02×10^7, 4.42×10^8cfu/ml and in *Tilapia zilli* body 4.92×10^6 ,4.26×10^7 ,4.44×10^8cfu/ml and in the gill show a mean population of 4.60×10^6 4.32×10^7 4.82×10^8 cfu/ml. *Oreochromis nilocticus* body 5.43×10^6, 5.90×10^7, and 3.96×10^8 were the gill show a mean population of 4.30×10^6, 5.36×10^7cfu/ml, and 4.80×10^8cfu/ml. The means population of *Leptocypris nilocticus* body 5.64×10^6, 4.72×10^7, and 4.8×10^8 cfu/ml as shown in Table 1.

Occurrence of Bacteria on Sampled Fishes

The following species of bacteria where found on *Clarias gariepinus, Staphylococcus aureus Pseudomonas* sp., *Proteus* sp., *Bacillus* sp. and *Micrococcus* sp. (Table 2) Also *Staphylococcus aureus Pseudomonas* sp, *Proteus* sp., *Bacillus* sp., *Micrococcus* sp. and *Klebsiella* sp. were the bacteria species found on *Tilapia zilli* (Table 3). In *Oreochromis nilocticus* the bacteria found were *Staphylococcus aureus. Pseudomonas* sp. *Bacillus* sp. And *Micrococcus* sp. (Table 4) and the following bacteria isolates were found on *Leptocypris nilocticus* sample *Bacillus* sp. *Pseudomonas* sp. and *Staphylococcus aureus* (Table 5). The result of the Gram stain, other biochemical test of the isolates and number of each bacterial isolate present in each fish samples are shown in Table 2, 3, 4 and 5 respectively.

Table 1: Bacteria colony count in body and gill of sampled fishes from Dangana Lake Lapai Nigeria.

Fish species	Sample area	Dilution factors	Mean ±S.E colony	Population in cfu/ml
Clarias gariepinus	Body	10^{-5}	49.00±3.58	4.90×10^6
		10^{-6}	48.20±4.15	4.82×10^7
		10^{-7}	47.00±1.48	4.7×10^8
	Gill	10^{-5}	51.00±4.67	5.10×10^6
		10^{-6}	50.20±3.68	5.02×10^7
		10^{-7}	44.20±1.68	4.42×10^8
Tillapia zilli	Body	10^{-5}	49.20±5.08	4.92×10^6
		10^{-6}	42.60±2.36	4.26×10^7
		10^{-7}	44.40±2.04	4.44×10^8
	Gill	10^{-5}	46.00±6.70	4.60×10^6
		10^{-6}	43.20±3.73	4.32×10^7
		10^{-7}	48.20±2.54	4.82×10^8
Oreochromis nilocticus	Body	10^{-5}	54.33±6.38	5.43×10^6
		10^{-6}		
		10^{-7}	59.00±3.78	5.90×10^7
			39.67±3.18	3.96×10^8
	Gill	10^{-5}	43.00±7.37	4.30×10^6
		10^{-6}	53.67±6.98	5.36×10^7
		10^{-7}	48.00±1.15	4.80×10^8
Leptocypris nilocticus	Body	10^{-5}	56.4±3.23	5.64×10^6
		10^{-6}		
		10^{-7}	47.2±1.77	4.72×10^7
			48±3.16	4.8×10^8

Table 2; Biochemical characteristic of isolates from body and gill of *Clarias gariepinus* sampled from Dangana Lake.

Code	Number of fish present	Gram Staining	Shape	Catalase	Coagulase	Indole	Methyl red	Citrate	Glucose	Sucrose	Fructose	Lactose	Probable organism
BODY	10	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	6	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp
	5	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
	4	-	Rod	+	-	-	+	+	AG	A	A	A	*Proteus* sp
GILL	6	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp
	10	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	5	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
	4	-	Rod	+	-	-	+	+	AG	AG	A	A	*Proteus* sp
	3	+	Cocci	-	-	+	-	+	AG	AG	A	NA	*Micrococcus* sp

KEY: + = Positive; - = Negative; AG= Acid and Gas production; A= Acid production; NA = No Acid and Gas production

Table 3: Biochemical characteristic of isolates from body and gill of *Tilapia zilli* sampled from Dangana Lake

Code	Number of fish present	Gram Staining	Shape	Catalase	Coagulase	Indole	Methyl red	Citrate	Glucose	Sucrose	Fructose	Lactose	Probable organism
BODY	8	-	Rod	+	-	-	+	+	AG	A	A	AG	*Klebsiella* sp.
	8	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	8	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
GILL	10	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	6	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
	6	+	Cocci	-	-	+	-	+	AG	AG	A	NA	*Micrococcus* sp
	6	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp

KEY: + = Positive; - = Negative; AG= Acid and Gas production; A= Acid production; NA = No Acid and Gas production

Table 4: Biochemical characteristic of isolates from body and gill of *Oreochromis nilocticus* sampled from Dangana Lake

Code	Number of fish present	Gram Staining	Shape	Catalase	Coagulase	Indole	Methyl	Citrate	Glucose	Sucrose	Fructose	Lactose	Probable organism
BODY	6	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	6	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp
	4	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
GILL	8	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp
	4	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*
	4	+	Cocci	-	-	+	-	+	AG	AG	A	NA	*Micrococcus* sp

KEY: + = Positive; - = Negative; AG= Acid and Gas production; A= Acid production; NA = No Acid and Gas production

Table 5: Biochemical characteristic of isolate from body of *Leptocypris nilocticus* sampled from Dangana Lake

Code	Number of fish present	Gram Staining	Shape	Catalase	Coagulase	Indole	Methyl red	Citrate	Glucose	Sucrose	Fructose	Lactose	Probable organism
Body	6	+	Rod	+	-	+	-	+	A	AG	AG	A	*Bacillus* sp
	6	-	Rod	+	+	-	+	+	A	NA	NA	NA	*Pseudomonas* sp
	3	+	Cocci	+	+	-	-	-	AG	A	A	AG	*Staphylococcus aureus*

KEY: + = Positive; - = Negative; AG= Acid and Gas production; A= Acid production; NA = No Acid and Gas production

Discussion

Freshly harvested aquaculture products, particularly those from tropical regions may harbour pathogenic bacteria, which form part of natural micro-flora of fish (Eze *et al.*, 2011). High population of bacterial colony may be due to discharge of waste into water bodies upon which the fish feeds on or it might result from flooding during raining season (Ajayi, 2012). The result of the present study revealed that *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp. *Proteus* sp. and *Micrococcus* sp were the bacteria species associated with fresh sampled fishes from Dangana lake lapai Nigeria. The present of *S. aureus* was attributed to contamination of sampled fish by man through handling and processing (Ibrahim *et al*., 2014, Clucas and Ward 1996). In a similar study carried out by Moshood and Tenghaziyamin (2012) *Bacillus* sp., *Proteus mirabilis* and *Klebsiella* sp. were found to be associated with fish, and it was suspected that organism may have contaminated the fish through human handlers, air, water and soil. Bacteria isolates from this finding are also similar with the finding of Ibrahim *et al*., (2014) who report the presence of *S. aureus Bacillus sp., Pseudomanas sp* and *Klebsiella* islolates from their work. The different species of bacteria isolated from this studied agrees with the finding of Ajayi (2012) which isolated *Staphylococcus aureus*, *Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp. and *Micrococcus* sp from their studies. The present of this organism is not surprising since Shinkafi and Ukwaja (2010) also reported that fish live in habitat full of microorganisms and confirmed that bacteria flora associated with Nigerian water culture include *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp. and others. In conclusion, this research has brought to light those bacterial species associated with freshwater fishes from Dangana lake Lapai Niger state and has shown that some are potentially pathogenic to humans. Hence there is need to adequately process fish before consumption.

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