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A comparative study on bacteria occurrence, diversity and population in fish organs and body parts

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Bacteria diseases are widespread and can be of particular importance in fish farming. Bacteria exist as micro flora in water until certain environmental conditions such as poor water quality occur, which could impose a stress on fish, thereby making them vulnerable to infection, most especially by pathogenic bacteria. This study was carried out to assess and compare the bacteria diversities and population in *Clarias gariepinus, Sarotherodon melanotheron* and *Oreochromis niloticus,* in the three grow out ponds of the aquaculture unit of the Department of Marine Science and Lagoon Front of the University of Lagos. It also aims at determining their public health significance. The experiment was carried out between May-September 2013. Water samples were collected from the three grows out ponds in the Department of Marine Sciences and from Lagos lagoon Front of the University of Lagos. In each case, water samples were analysed for the possible indicator organisms of faecal and industrial pollutions such as *Escherichia coli, Staphylococcus aureus, Salmonella* spp*., feacal streptococcus, Vibrio* spp., and *Clostridium* spp. From the result obtained, all the bacteria listed above were first seen as common bacteria in all the samples of water analysed. Further studies (biochemical tests) also reveal bacteria such as Klebsiella spp., *Proteus* spp., *Enterobacter aerogenes, Citrobacter* spp*. and Pseudomonas aeroginosa.* Moreover, the same indicator bacteria seen in water samples were also detected in the different body parts (flesh, mouth, gill and gut) of each of the fish species analysed, both from the Departmental ponds and Lagoon front, except *Clostridium perfringes* (*C. welchii*), *Vibrio cholera and Salmonella typii* which were found in the gut of those fish species from the departmental ponds A-C and in the mouth, gill and gut of the fishes from lagoon front. The population of each of these bacteria was found to be highest in the gut region, followed by the gill, the mouth and least in the flesh. There was no significant difference in the population of each of the bacterial across pond water (P>0.05). Same is the case with each bacteria analysed across body parts of the fish species (skin, mouth, gill and gut) in the Departmental ponds, that is, no significant difference (P>0.05). But, when compared with the lagoon front (both for water and fish samples) the difference was highly significant (P<0.05). None of the population of the bacteria in the ponds exceeds the limit for human consumption. The bacteria load in the lagoon fish (skin, mouth, gill and gut) was higher than the recommended limit for human consumption. Therefore, they are not fit for consumption most especially samples from the mouth, gill and gut, except effective processing treatment is employed before consumption. Due policy should also be taken by the government to curtail the tradition of indiscriminate discharge of untreated effluent into the lagoon.

Key words: Bacteria load, *Clarias gariepinus, Sarotherodon melanotheron* and *Oreochromis niloticus,* Lagos lagoon.

INTRODUCTION

Fish is one of the cheapest sources of animal protein available all over the world for human consumption. Fish,

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among all other important protein food stuff such as eggs, milk, meat, and other product constitute an excellent source of protein of high biological value, (Cleube, 2008). It was also observed that freshwater fish represent an important source of animal protein to human nutrition. However, the challenge due to pathogenic organisms especially bacteria has limited its effective production and availability.

Disease occurrence in aquatic animal production is beginning to show a significant impact on yield (Hudson, 1990). In a situation where there is low stocking densities, with low management practice characterized by traditional captured fisheries or extensively managed culture system, there was low yield levels (Sherman et al., 2000). In effect, the rate of disease occurrence was low. On the other hand, as aquaculture intensifies, necessitating fast movement of aquatic species in association with their pathogens, disease level has been triggered (Olufemi, 1998). The possible economic losses a fish farmer may suffer in the event of disease occurrence are mortality, growth reduction during and after an outbreak, treatment or prevention expenses, loss of investor's confidence. Loss or damage to brood stock may have major consequences on the genetic pool, increase in the time required for the fish to reach market weight-size and therefore postponement or loss of the opportunity to sell fish. Also, damage to wild population may result not only in the loss of a resource but also decrease biodiversity and a shift in the ecological balance (Cameron and Douglas, 2002). The good knowledge of fish disease agent is needed to prevent, cure, or minimise those negative effect.

Moreover, of all fish diseases, bacterial diseases are widespread, and can be of serious concern in fish farming. This has been responsible for heavy mortality in both wild and cultured fish (Hudson, 1990). Disease caused by bacteria are often chronic than acute and may also cause a high percentage of death which is highly induced by environmental stress (Olufemi, 1998).

Pathogens living in the fish depend on the type and abundance of microorganisms present in the water in which they live (Cahill, 1990, cited by Atlas and Bartha, 1998). Cahill also observed that the range of bacteria genera isolated from eggs, skin, gills, and intestine is related to the aquatic habitat of the fish and varies with factors such as salinity of the habitat and the bacteria load in the water. Bacteria recovered from the skin and gills may be transient rather than resident on the gill surface. Micro floral of fish intestine often appeared to vary with complexity of the fish digestive system (Cahill, 1990, cited by Atlas and Bartha, 1998). The bacteria genera present in the gut generally seem to be those from the environment or diet which can survive and multiply in the intestinal tract, although there is evidence for a distinct intestinal micro floral in some species (Cahill, 1990, cited by Atlas and Atlas and Bartha, 1998). Obligate anaerobes have also been recovered from Tilapia and carp intestine (Cahill, 1990, cited by Atlas and Bartha, 1998).

However, some of these micro-organisms are not pathogenic, but those that are pathogenic can cause serious damage to fish such as Aeromonas salmonicida which causes furunculosis of salmonids, carp erythrodarmatitis and gold fish ulcer diseases; and man when consumed infected fish. For instance, cholera caused by *Vibrio cholera*; *salmonellosis*, caused by gram negative rod, *Salmonella spp; shigellosis*, caused by *Shigella dysentariae*, *Shigella flexneri, Shigella boydii, and Shigella sonnei*; *tuberculosis,* caused by *Mycobacterium tuberculosis* and dysentery caused by *Escherichia coli* (Okafor, 1985; Austin and Austin, 2007).

The aim of this study is to assess and compare bacteria occurrence, diversity and population in fish organs (gill and gut) and body part (buccal cavity and flesh) of cultured *Clarias gariepinus, Oreochromis niloticus and Sarotherodon melanotheron* in an intensive fish production system at the Aquaculture Unit of the Department of Marine Sciences, and Lagoon Front of the University of Lagos, Lagos, Nigeria. It also aims at providing information on the morphometrics of these fish species.

MATERIALS AND METHODS

Study area and sample collection

The study areas are the Aquaculture Unit of the Department of Marine Sciences, and Lagoon Front of the University of Lagos.

Water and fish samples were collected from the three grow-out ponds of the Aquaculture Unit of the Department of Marine Science and from the Lagoon Front of the University of Lagos. All samples were collected aseptically using sterile sample bottles for water. Sterile dissecting instrument were also used to take samples from the flesh, mouth, gill and gut of the fishes' body for analysis.

Morphometrics

Morphometric parameters of the fish samples such as weight, standard length, head length, gill length and buccal depth were measured with the use of top loading balance for weights and graduated measuring ruler for lengths. This was done for all the samples of fish taken from each pond and lagoon, and their values were recorded in two decimal places.

Preparation of the serial dilutions

90 to 100 ml of distilled water was dispensed into conical flask as diluents for each sample and 9 ml of these diluents was dispensed into MacCartney bottles for serial dilutions. The diluents were

autoclaved at 121°C for 15 min. Nutrients agar was also autoclaved along with the diluents, and both were kept to cool.

Water samples

1 ml was aseptically taken from the raw water sample into 9 ml distilled water in the MacCartney bottle to give dilution 10^{-1}. Fivefold serial dilutions was made using sterile pipette (10^{-1} to 10^{-5} serial dilutions) while raw water sample remained 10°. However, further dilutions till 10^{-9} were made where required so as to allow easy colony counting.

Fish samples

Using a sterile dissecting tools, mortar and pestle, samples was taken from the fish (flesh, gill, mouth and gut). Each sample was pounded into pieces and properly mixed together. Each of these samples was added into 9 ml sterile distilled water in the MacCartney bottle and thoroughly mixed together to make a dilution 10^{-1}. Fivefold serial dilution was made using sterile pipette (10^{-1} to 10^{-5}). However further dilution till 10^{-9} was made and used where required so as to allow easy colony counting.

Inoculation into the solid medium

1 ml of inoculums was pipette into sterile Petri dishes. This was done in duplicates and also labelled sequentially. Using pour plate method, about 15 ml of sterilized molten Nutrient agar medium, cooled to about 45°C was poured into the inoculated Petri dishes within 15 min of original dilution. Both the sample dilution and agar medium were mixed thoroughly and uniformly, and allowed to gel. Some plates were also prepared as control to check on the sterility of the diluents, glasswares and agar medium. The possibility of air contamination was also assessed with the use of control plates. All poured Petri dishes were incubated in inverted position at 37°C for 24 h.

Using the same procedure described above for the total bacteria count with nutrient agar as a general purpose medium, the following list of indicator bacteria of fecal and industrial pollution were also isolated from the water and fish organs (flesh, mouth, gill and gut) using their respective selective medium: Coliform bacteria and *E. coli* were isolated with MacConkey agar, *Staphylococcus* spp were isolated with Mannitol salt agar, *Salmonella* spp and *Shigella* spp were isolated with *Salmonella Shigella* agar (SSA), *Vibrio* spp were isolated with thiosulphate citrate bile salt agar (TCBS), *Streptococcus fecalis* were isolated with blood agar whose 5% is horse blood and lastly, *Clostridium* spp were isolated with reinforced Clostridium agar (RCA).

Colonial and microscopic examination

From the isolated colonies, the colonial characteristics were first determined with the colony counter magnifying lens, which was also used to count the numbers of colony in each plate. Further clarification was then conducted with the use of a light microscope, especially morphological characteristics. The shape and arrangement, and some other characteristics of the colonies were examined and recorded. Also Gram's staining was carried out according to Fawole and Oso (1988).

Biochemical test

The following biochemical tests were carried out and used to further identify the bacteria isolated and also to identify any other bacteria that could be present.

Catalase test

A drop of 3% hydrogen peroxide was placed on the centre of a slide and sterile wire loop was used to pick small portion of the micro-organism to be identified from nutrient agar plate into the hydrogen peroxide for immediate gas bubble formation. Quick Gas bubble or foaming indicates positive result (Olutiola et al., 1991).

Coagulase test

A drop of physiological saline was placed on two separate slides. A colony of the test organism was emulsified in each of the drop to make suspension. A drop of plasma was then added and mixed gently with the suspension. Clumping (due to coagulation) of the organisms in 10 s when viewed under the microscope indicate positive result (Olutiola et al., 1991). This was done for the plate suspected to be *Staphylococcus aureus.*

Motility test

A loopful of growth was inoculated into peptone water broth and incubated overnight. A wet preparation from the peptone water culture was prepared and examined under a microscope at x40 objective lens. Dating movement of the organism indicate a positive result (Olutiola et al., 1991).

Citrate utilization test

A slant of a citrate agar was aseptically inoculated with the organisms to be identified using a sterile wire loop. The inoculated citrate agar slant was incubated at 37°C for 24 h and observes the colour change daily for up to 4 days. Blue colouration indicate positive test (Olutiola et al*.,* 1991).

Indole reaction test

The micro-organisms to be identified were inoculated into tryptone broth for 48 h at 37°C, 5 drops of Kovac's reagent was then added. A deep red colour indicates positive result (Olutiola et al*.,* 1991).

Sugar fermentation test

Peptone water (7.5 g) was weight and diluted to 500 ml with distilled water after which few pinch of phenol red was added. 9 ml of broth was distributed into test tubes with Durham tubes inverted into each tube. The tubes were sterilized at 121°C (at 15 pounds pressure) for 15 min. 1% (w/v) aqueous solution of Glucose, Sucrose, Lactose, and Mannitol were prepared separately and sterilised. 1 ml of 1% of sugar solution was added aseptically using sterile pipette into each of the test tube containing 9 ml broth. The test organisms were inoculated into each set of test tubes. Uninnoculated test tubes serve as control. Incubation was done at 35°C for 5 days. A change in the initial colour of the solution indicates acid production, and gas in the inverted Durham tubes indicates gas production. The colour change is from red to yellow (Olutiola et al., 1991).

Oxidase test

A drop of a freshly prepared oxidase reagent was added onto a strip of filter paper. A little of the test organism was rubbed into it.

Table 1. Bacteria isolated from pond A to C and lagoon water.

Types of bacteria	Pond A	Pond B	Pond C	ULF
Salmonella typi	ND[1]	ND[1]	ND[1]	DE[1]
Shigella spp	DE	DE	DE	DE
Escherichial coli	DE	DE	DE	DE
Streptococcus fecalis	DE	DE	DE	DE
Vibrio cholera	ND[1]	ND[1]	ND[1]	DE[1]
Clostridium perfringes	ND[1]	ND[1]	ND[1]	DE[1]
Enterobacter aerogene	DE	DE	DE	DE
Proteus spp	DE	DE	DE	DE
Klebsiella spp	DE	DE	DE	DE
Pseudomonas Aeroginosa	DE	DE	DE	DE

DE – Detected; N.D – Not detected; ND[1] – Not detected but other strains/spp were seen; DE[1] – Detected along with other strains/spp; ULF- UNILAG lagoon front.

Colour changes into deep-blue in 5 s indicate positive test while non colouration indicate negative test.

Test for the production of hydrogen sulphite and Indole using Sulphite Indole Motility medium (SIM medium)

15 g of SIM agar was suspended in 500 ml of distilled water (30 g in 1 L). It was brought to boil to dissolve completely. It was mixed well and distributed into test tubes and tacked with cotton wool and aluminium foil. It was then sterilized by autoclaving at 121°C for 15 min and allowed to set/solidified. With the use of a straight wire/inoculating needle, the organism was inoculated into the SIM medium and incubated at 37°C. After 24 h, sulphite production (Blackening of the medium) was noticed. Also when 1 ml of Kovac's reagent was added, a red colouring of the surface layers within 10 min indicates the presence of indole (Cheesbrough, 1984).

Statistical analysis

The results of bacteria isolates (populations) and morphometrics were subjected to a one-way analysis of variance (ANOVA). The significant level was $p<0.05$.

RESULTS

Bacteria isolated from water and fish samples

The organisms (bacteria) found in all the water samples analysed in Ponds A-C at the Aquaculture Unit of the Marine Science Department and the Lagoon Front of the University of Lagos are presented in Table 1. It shows the presence of the following indicator organisms of faecal, industrial and other sources of pollution such as *S. aureus, Salmonella* spp*, Shigella spp, faecal streptococcus, Clostridium spp, E. coli and Vibrio spp*. Morphological and biochemical examination also revealed other organisms such as *Klebsiella spp, Proteus spp, Citrobacter spp, Enterobacter aerogenes and P. aeruginosa.*

It was observed that the same types of bacteria isolated from the ponds were also found in the Lagoon, except *Clostridium perfringes, V. cholera* and *Salmonella typhi.*

Tables 2 and 3 also show the bacteria found in the flesh, mouth (Buccal cavity), gill and gut of each of the three species of fish analysed, both in the Departmental pond and lagoon front. The same type of bacteria were found in all the organs that were analysed both in the Departmental pond and lagoon front except *C. perfringes*, *V. cholera and S. typhi* which were seen in the lagoon fish species (mouth, gill and gut only), and guts of the fishes from the Department. The result of these bacteria load shows a marked difference ($P<0.05$) between the population of organisms found in the departmental ponds (A to C) and those found in the UNILAG Lagoon front.

The results of the bacterial population in the fish organs from the pond in comparison with that obtained from lagoon front species are shown in Table 7 to 10. The result of the population of bacteria in the buccal cavity (mouth) of *C. gariepinus, O. niloticus* and *S. melanotheron* harvested from the UNILAG lagoon front as compared with those from the departmental pond were shown in Table 7. The result show a significant ($P<0.05$) difference between the population of bacteria in the *C. gariepinus* harvested from the lagoon front when compared with those in the ponds. Similar trend was also observed in *O. niloticus* and *S. melanotheron* harvested in the ponds and UNILAG lagoon front. The situation was the same with the flesh, mouth, gill and gut of all the three fish species harvested from UNILAG lagoon front when compared with those in the Departmental ponds. There was a marked difference ($P<0.05$) between the populations of bacteria in the pond fish organs when compared with those from UNILAG lagoon front (Table 8 to 10).

Colonial, morphological and biochemical characteristics of bacteria diversity in ponds and lagoon front

Table 4 shows the colonial, morphological, and biochemical characteristics of various bacteria found in

Table 2. Bacteria isolated from the three species of fish in Pond A-C.

Types of bacteria	Pond A				Pond B				Pond C			
	FL	MT	GL	GT	FL	MT	GL	GT	FL	MT	GL	GT
Salmonella typi	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE
Shigella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
E. coli	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Streptococcus fecalis	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Vibrio cholera	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE
Clostridium perfringes	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE	ND[1]	ND[1]	ND[1]	DE
Enterobacter aerogene	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Proteus spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Klebsiella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Pseudomonas aeroginosa	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE

DE – Detected; N.D –Not detected; ND[1] – Not detected but other strains were seen; DE[1] – Detected along with other strains/spp; FL- Flesh; MT - Mouth; GL- Gilll; GT- Gut.

Table 3. Bacteria isolated from the three species of fish from the lagoon front.

Types of bacteria	ULF Fish A				ULF Fish B				ULF Fish C			
	FL	MT	GL	GT	FL	MT	GL	GT	FL	MT	GL	GT
Salmonella typi	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE
Shigella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
E. coli	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Streptococcus fecalis	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Vibrio cholera	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE
Clostridium perfringes (C. welchii)	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE	ND[1]	DE	DE	DE
Enterobacter aerogeneS	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Proteus spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Klebsiella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Pseudomonas eroginosa	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE

DE – Detected; N.D – Not detected; ND[1] – Not detected but other strains/spp were seen; DE[1] – Detected along with other strains/spp; FL - Flesh; MT – Mouth; GL- Gilll; GT- Gut.

ponds and lagoon front (both in water and fish samples). It shows their edges, colour, elevations, shape and arrangement. This table also shows various identification procedures, tests and techniques by which several of these bacteria species were identified. It was also used to determine the presence of pathogenic bacteria. Some of this test includes: gram's staining, catalase test, coagulase test, citrate utilization test, sugar fermentation test, oxidase test, indole, motility and test for sulphite production (as outlined on the table).

Among the enteric gram negative rods, organisms such as: *E. coli, Klebsiella spp, Enterobacter spp, Citrobacter spp,* are lactose fermenters, giving a positive reaction to lactose by producing acid and gas while their non lactose fermenter counterparts such as *Salmonella, Shigella*, *Proteus* gave a negative reaction to lactose and some other sugars, except glucose (and mannitol for *S. typhi*) fermentation but without gas production (Table 4). Other species of *salmonella* were seen to ferment mannitol and glucose with gas production.

The two sets of organisms discussed above are members of a family known as *Enterobacteriaceae* and they are all oxidase negative, that is, they do not produce a deep purple or blue colouration during oxidase test (Table 4). *P. aeroginosa* and *Vibrio spp* are oxidase positive (as shown in the Table 4) and do not belong to this family. They do not react positively to lactose test. Tests such as oxidase, citrate utilization, catalase and some colonial characteristics like colour, elevation, shape and edges will be sufficient and thus were used to identify them.

Moreover, gram positive cocci and rods are also identified and outlined in Table 4, for instance, Cocci such as *staphylococcus* is catalase positive while *Streptococcus* (e.g *faecal streptococcus*) is catalase negative (Table 4). Catalase test was used to differentiate staphylococcus from streptococcus spp, while coagulase test was used to differentiate *S. aureus* from other *Staphylococcus species*.

Table 4. Colonial, morphological and biochemical characteristics of various bacteria isolated from ponds A to C in the Aquaculture unit of the Department of Marine Science and Lagoon front of the University of Lagos.

Edge	Colour	Elevation	Shape and arrangement	Gram stain	Catalase	Coagulase	Oxidase	Citrase utilization	Glucose	Lactose	Sucrose	Mannitol	SIM medium			Probable organisms (Bacteria)
													H_2S	Indole	Motility	
Regular.	Colourless with black centre	Raised -	Short Rod	-	+	-	-	-	A	-	-	A	+	-	+	*Salmonela typi*
Regular	Colourless with black centre	-	Short rods	-	+	-	-	+	AG	-	-	AG	+	-	+	*Other Salmonella spp*
Irregular	Yellow	Raised	Cocci in cluster	+	+	+	NR	NR	A	A	A	A	-	-	-	*Staphilicoccus aureus*
Undulated	Red	Flat	Rod in singles	-	+	-	-	+	AG	AG	AG	AG	-	+	+	*Escherichial coli*
Regular	Colourless	convex	Rod	-	+	-	-	-	A	-	-	-	-	-	-	*Shigella spp*
Regular	Yellow	Raised	Curved rod	-	+	-	+	+	A	-	A	A	-	+	+	*Vibrio cholera*
Regular	Green	Raised	Curved rod	-	+	-	+	+	A	-	-	A	-	+	+	Other *Vibrio spp*
Irregular	Cream	Raised	Rod in chain	+	-	+	NR	NR	A	A	-	AG	-	-	-	*Clostridium perfringes (Welchii)*
Irregular	Cream	Raised	Rod in chain	+	-	+	NR	NR	A	-	-	AG	-	-	+	*Clostridium spp*
Irregular	Red	Raised	Cocci in short chain	+	-	-	NR	NR	AG	AG	AG	AG	NR	NR	-	*Feacal streptococcus*
Undulated	Cream	Slightly raised	Rod in pairs and singles	-	-	+	-	+	AG	AG	AG	AG	-	-	-	*Klebsiella spp*
Undulated	Cream	Flat	Rod	-	+	-	-	+	A	-	-	-	+	-	+	*Proteus mirabilis*
Serrated	Cream pink	Raised	Rod	-	+	-	-	+	AG	AG	AG	AG	-	-	+	*Enterobacter aerogenes*
Regular	Red	Raised	Rod	-	+	-	-	+	AG	AG	-	AG	+	-	+	*Citrobacter spp*
Irregular	Blue green	Raised	Rod	-	+	-	+	+	A	-	-	-	-	-	+	*Pseudomonas aeruginosa*

SIM- Sulphite, Indole and motility test medium, H2S- Hydrogen sulphite, NR-Not Required, AG- Acid and Gas production, A-acid production.

Gram positive rods such as *C. perfringes* was also identified and other *Clostridium* genera which could not be identified to species level were also seen. *C. perfringes* was identified and singled out from other members of *Clostridium* by its lack of motility and its negative reaction to Lactose fermentation test.

V. cholera was also differentiated from other *Vibrio spp* identified in the TCBS plate (Thiosulphate citrate-bile salt sucrose agar plate) by its ability to ferment sucrose and its characteristic yellow colony on TCBS agar plate.

S. typhi was also identified among other *Salmonella spp* seen on the agar plate by its ability to ferment mannitol and glucose but without gas production whereas other species of *salmonella* do ferment glucose and mannitol with the release of gas.

Morphometric parameters

The result of the morphometric parameter revealed that there is no significant difference in the standard length of *O. niloticus* in pond B, *S. melano*theron in pond C and *O. niloticus* in Lagoon front (Table 5).

For the head length, there is no significant difference between *S. melanotheron* in pond C, *O.*

Table 5. Result of the morphometric parameter (Mean±SD).

Parameter	Pond A	Pond B	Pond C	ULF A	ULF B	ULF C
Weight (g)	276.72±3.91^{d}	33.04±2.15^{a}	46.08±2.15^{b}	376.72±3.91^{e}	48.15±3.65^{b}	54.19±2.43^{c}
Standard Length (cm)	31.35±0.98^{d}	9.85±0.17^{a}	11.30±0.35^{a}	30.52±3.56^{d}	10.60±0.69^{a}	13.75±0.87^{b}
Head Length (cm)	9.55±0.06^{c}	2.95±0.06^{a}	4.05±0.06^{b}	9.24±1.45^{c}	3.95±0.17^{b}	3.35±0.17^{b}
Gill length (cm)	8.33±1.27^{b}	2.42±0.14^{a}	2.85±0.06^{a}	8.30±1.27^{b}	2.88±0.03^{a}	2.35±0.17^{a}
Buccal Depth(cm)	7.25±0.29^{b}	1.75±0.29^{a}	2.35±0.11^{a}	6.68±1.07^{b}	2.30±0.23^{a}	1.78±0.14^{a}

ULF- UNILAG Lagoon front; A - *Clarias gariepinus;* B - *Oreochromis niloticus;* C - *Sarotherodon melanotheron.*

Table 6. Bacteria load in ponds and lagoon (cfu/ml±SD) × 10^{5}.

Types of organisms	Pond A	Pond B	Pond C	UNILAG lagoon front
TCC	0.52±0.02^{b}	0.39±0.01^{a}	0.36±0.01^{a}	90.63±0.04^{c}
Total plate count	5.90±0.14^{c}	5.55±0.07^{b}	3.85±0.07^{a}	958.00±0.02^{d}
Staphylococcus aureus	0.09±0.01^{a}	0.07±0.01^{a}	0.05±0.01^{a}	14.10±0.14^{b}
Escherichia coli	0.05±0.01^{a}	0.03±0.01^{a}	0.02±0.01^{a}	48.03±0.04^{b}
Faecal streptococcus	0.07±0.12^{a}	0.03±0.09^{a}	0.09±0.08^{a}	24.75±0.07^{b}
Salmonella spp	0.09±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}	29.40±0.14^{b}
Shigella spp	0.04±0.01^{a}	0.02±0.01^{a}	0.04±0.01^{a}	59.45±0.21^{b}
Vibrio spp	0.06±0.02^{a}	0.05±0.01^{a}	0.40±0.00^{a}	50.63±0.24^{b}
Clostridium spp	0.01±0.01^{a}	0.02±0.03^{a}	0.02±0.01^{a}	28.35±0.12^{b}

Mean±S.D with superscript of the same alphabet either 'a' or 'b' shows no significant difference (P>0.05); Mean ± S.D. with superscript of different alphabet e.g 'ab' 'b' 'c' 'd' or 'e' shows that there was significant difference (P<0.05). ULFA- lagoon front fish A (*Clarias gariepinus*); ULFB- lagoon front fish B (*Oreochromis niloticus*); ULFC-lagoon front fish C (*Sarotherodon melanotheron)*; TCC- Total coliform count.

niloticus and *S. melanotheron* in the lagoon front.

For the gill length and buccal depth, there is no significant difference between *C. gariepinus* from pond A and *C. gariepinus* from lagoon front. The same goes for the *O. niloticus* and *S. melanotheron* in both Pond B and C, when compared with those in the lagoon front (P>0.05).

DISCUSSION

This study shows the isolation of various bacteria cells from three major fish species of commercial importance namely: *C. gariepinus, S. melanotheron and O. niloticus* which were harvested from the three grow out ponds of the Aquaculture Unit of the Department of Marine Sciences and Lagos Lagoon Front of the University of Lagos.

Seven, among the enteric gram negative rods and gram positive cocci and rods were investigated which includes*: E. coli*, *Salmonela spp, Shigella spp, Vibrio spp, S. aureus,* feacal *Streptococcus,* and *Clostridium spp*. However, during the biochemical tests, other bacteria such as *Klebsiella spp, Citrobacter spp, Proteus spp, P. aeroginosa, and Enterobacter spp* were also discovered. From the study so far, and with reference to Table 6, it could be deduced that the highest concentration of these bacteria is in the lagoon front when compared with the Departmental ponds. Also, the highly infested part in all the three fish species was gut, especially those one from the lagoon front. This was followed by the gill and mouth, while the least population was found in the flesh (Table 7 to 10).

However, the highest microbial load of each bacteria species as well as their total coliform count obtained in the pond fishes was less than x10^{6} count, except the total plate count of the gut which is 3.70±0.02x10^{6} for *C. gariepinus*, 3.30±0.07×10^{6} for *O. niloticus* and 2.81±0.04×10^{6} for *S. melanotheron*; while gill recorded total plate count of 8.88±0.02x10^{5} for *C. gariepinus*, 7.71±0.13x10^{5} for *O. niloticus* and 9.68±0.03x10^{5} for *S. melanotheron*. Total plate count for buccal cavity is 4.45±0.07×10^{4} for *C. gariepinus*, 5.55±0.21×10^{4} for *O. niloticus* and 5.10±0×03×10^{4} for *S. melanotheron*. Bacterial load of flesh samples (Total Plate Count) are 1.40±0.02x10^{3} for *C. gariepinus*, 1.35±0.07×10^{3} for *O. niloticus* and 1.95±0.04×10^{3} for *S. melanotheron*. The result of the bacterial population in the gill, buccal cavity and flesh samples of departmental pond fishes are within the acceptable limit according to ICMF (1986).

Moreover, the microbial load of these same species of fish in the lagoon front differs. The total plate count of the gut is 2.96±0.04x108 for *C. gariepinus*, 2.82±0.04×108 for *O. niloticus* and (2.92±0.04×108) for *S. melanotheron;*

Table 7. Bacteria load in the flesh of *Clarias gariepinus, Oreochromis niloticus and Sarotherodon melanotheron* (Ponds and Lagoon) (cfu/g±SD) × 10^3.

Type of organisms	FPCG	FLCG	FPON	FLON	FPSM	FLSM
Total coliform count	0.13±0.01^a	8.19±0.011^d	0.15±0.01^a	7.27.50±0.04^c	0.12±0.02^a	6.47.50±0.04^b
Total plate count	1.49±0.02^a	60.25±0.35^e	1.35±0.07^a	26.72±0.03^d	1.95±0.04^b	15.7±0.02^c
Staphylococcus aureus	0.02±0.00^a	0.8±0.03^c	0.02±0^a	0.42±0.04^b	0.03±0.00^a	0.43±0.05^b
Escherichial coli	0.01±0.00^a	0.74±0.03^a	0.03±0.00^a	0.70±0.01^b	0.03±0.00	0.17±0.02
Faecal streptococcus	0.01±0.00^a	0.16±0.01^a	0.01±0.00^a	0.29±0.01^b	0.01±0.00	0.14±0.01
Salmonella spp	0.02±0.00^a	0.28±0.01^d	0.01±0.00^a	0.18±0.01^c	0.02±0.01^a	0.11±0.01^b
Shigella spp	0.01±0.00^a	0.70±0.01^d	0.02±0.00^a	0.57±0.01^c	0.03±0.00^a	0.38±0.04^b
Vibrio spp	0.01±0.00^a	0.13±0.01^b	0.01±0.00^a	0.39±0.01^d	0.02±0.00^a	0.28±0.01^c
Clostridium spp	0.01±0.00^a	0.81±0.01^c	0.01±0.00^a	0.77±0.02^b	0.01±0.00a	0.84±0.01d

Mean±S.D with superscript of the same alphabet either 'a' or 'b' shows no significant difference (P>0.05); Mean±S.D. with superscript of different alphabet e.g 'ab' 'b' 'c' 'd' or 'e' shows that there was significant difference (P<0.05). FPCG- Fish pond *Clarias gariepinus*; FLCG- flesh lagoon *Clarias gariepinus*; FPON- flesh pond *Oreochromis niloticus*; FLON- Flesh lagoon *Oreochromis niloticus*; FPSM- Flesh pond *Sarotherodon melanotheron;* FLSM- Flesh lagoon *Sarotherodon melanotheron*.

Table 8. Bacteria load in the Bucca cavity (mouth) of *Clarias gariepinus, Oreochromis niloticus and Sarotherodon melanotheron* (Ponds and Lagoon) (cfu/g±SD) x 10^4.

Type of organism	MPCG	MLCG	MPON	MLON	MPSM	MLSM
Total coliform count	3.75±0.02^b	40.20±0.04^f	4.85±0.07^c	23.30±0.22^d	2.40±0.28^a	37.00±0.00^e
Total plate count	4.45±0.07^a	137.00±0.01^e	5.55±0.21^b	129.00±0.21^d	5.10±0.03^b	111.00±0.04^c
Staphylococcus aureus	0.14±0.01^a	2.95±0.21^c	0.17±0.02^a	2.95±0.00^c	0.16±0.02^a	2.49±0.02^b
Escherichial coli	0.54±0.04^a	4.95±0.01^d	0.88±0.06^b	2.15±0.07^c	0.57±0.05^a	2.12±0.02^c
Fecal streptococcus	0.33±0.08^a	2.05±0.07^e	0.64±0.01^b	1.25±0.07^c	0.42±0.06^a	1.91±0.01^d
Salmonella spp	0.36±0.04^a	5.95±0.02^e	0.52±0.05^b	4.15±0.00^d	0.73±0.04^c	6.08±0.04^f
Shigella spp	0.21±0.04^a	4.18±0.04^c	0.60±0.13^b	5.15±0.07^d	0.44±0.08^b	5.65±0.07^e
Vibrio spp	0.37±0.03^a	2.31±0.02^c	0.57±0.04^b	3.95±0.03^d	0.68±0.06^b	5.30±0.14^e
Clostridium spp	0.55±0.08^a	4.15±0.07^d	0.65±0.04^{a,b}	2.89±0.02^c	0.68±0.03^b	6.25±0.07^e

Mean±S.D with superscript of the same alphabet either 'a' or 'b' shows no significant difference (P>0.05); Mean±S.D. with superscript of different alphabet e.g 'ab' 'b' 'c' 'd' or 'e' shows that there was significant difference (P<0.05). MPCG- Mouth pond *Clarias gariepinus*; MLCG- Mouth lagoon *Clarias gariepinus*; MPON-Mouth pond *Oreochromis niloticus*; MLON- Mouth lagoon *Oreochromis niloticus*; MPSM- Mouth pond *Sarotherodon melanotheron*; MLSM- Mouth lagoon *Sarotherodon melanotheron.*

Table 9. Bacteria load in the gill of *Clarias gariepinus, Oreochromis niloticus and Sarotherodon melanotheron* (Ponds and Lagoon) (cfu/g±SD) x 10^5.

Type of bacteria	GPCG	GLCG	GPON	GLON	GPSM	GLSM
Total coliform count	4.73±0.04^a	58.75±0.07^e	5.81±0.02^c	43.20±0.28^d	5.46±0.64^b	67.25±0.71^f
Total plate count	8.88±0.02^b	186.00±0.14^e	7.71±0.13^a	152.00±0.09^d	9.68±0.03^c	163.00±0.05^d
Staphylococcus aureus	0.40±0.04^b	4.95±0.07^d	0.24±0.01^a	5.95±0.07^e	0.24±0.01^a	4.05±0.03^c
Escherichia coli	0.76±0.05^b	6.25±0.08^e	0.59±0.01^a	2.85±0.03^c	0.75±0.06^b	3.65±0.07^d
Faecal streptococcus	0.76±0.17^a	3.65±0.07^c	0.31±0.04^a	3.25±0.07^b	0.41±0.05^a	3.15±0.07^b
Salmonella spp	0.27±0.05^a	5.65±0.07^d	0.26±O.O2^a	2.35±0.19^b	0.16±0.02^a	3.95±0.04^c
Shigella spp	0.16±0.04^a	3.40±0.00^d	0.37±0.04^b	3.65±0.07^e	0.30±0.01^b	3.05±0.07^c
Vibrio spp	0.20±0.01^a	1.95±0.17^b	0.32±0.01^a	1.98±0.01^b	0.31±0.03^a	2.75±0.00^c
Clostridium spp	0.30±0.05^a	1.88±0.07^c	0.32±0.06^a	1.75±0.07^b	0.46±0.05^a	3.15±0.07^d

Mean±S.D with superscript of the same alphabet either 'a' or 'b' shows no significant difference (P>0.05); Mean±S.D. with superscript of different alphabet e.g 'ab' 'b' 'c' 'd' or 'e' shows that there was significant difference (P<0.05). GPCG- Gill pond *Clarias gariepinus*; GLCG- Gill lagoon *Clarias gariepinus*; GPON- Gill pond *Oreochromis niloticus*; GLON- Gill lagoon *Oreochromis niloticus*; GPSM- Gill pond *Sarotherodon melanotheron;* FLSM- Gill lagoon *Sarotherodon melanotheron.*

Table 10. Bacteria load in the Gut of *Clarias gariepinus, Oreochromis niloticus and Sarotherodon melanotheron* (Ponds and Lagoon) (cfu/g±SD) × 10^5.

Type of bacteria	GTPCG	GTLCG	GTPON	GTLON	GTPSM	GTLSM
Total coliform count	9.68±0.04^b	125.49±0.02^d	8.22±0.03^a	127.22±0.04^e	9.85±0.02^c	135.57±0.06^f
Total plate count	37.00±0.02^c	2960.00±0.04^f	33.00±0.07^b	2820.00±0.04^d	28.00±0.04^a	2920.00±0.04^e
Staphylococcus aureus	3.58±0.04^b	70.83±^{a}0.04^d	2.90±0.00^a	27.30±0.28^c	3.49±0.01^b	27.37±0.05^c
Escherichia coli	2.15±0.07^a	55.57±0.05^f	3.45±0.07^c	26.43±0.04^d	2.84±0.09^b	26.68±0.03^e
Faecal streptococcus	2.45±0.07^a	62.25±0.07^e	2.83±0.11^b	17.03±0.04^c	2.69±0.01^b	19.37±0.05^d
Salmonella spp	2.10±0.14^b	39.45±0.07^f	2.88±0.04^c	16.19±0.01^d	1.37±0.05^a	25.030.04^e
Shigella spp	1.43±0.04^a	78.03±0.04^e	3.85±0.08^b	51.03±0.04^c	1.33±0.04^a	51.18±0.03^d
Vibrio spp	2.82±0.04^c	40.06±0.08^f	1.47±0.04^a	24.04±0.05^d	1.85±0.07^b	35.03±0.04^e
Clostridium spp	1.38±0.04^a	67.17±0.04^f	2.95±0.07^b	58.05±0.07^e	3.85±0.07^c	48.08±0.01^d

Mean±S.D with superscript of the same alphabet either 'a' or 'b' shows no significant difference (P>0.05); Mean±S.D. with superscript of different alphabet e.g 'ab' 'b' 'c' 'd' or 'e' shows that there was significant difference (P<0.05). GTPCG- Gut pond *Clarias gariepinus*; GTLCG- Gut lagoon *Clarias gariepinus*; GTPON- Gut pond *Oreochromis niloticus*; GTLON- Gut lagoon *Oreochromis niloticus*; GTPSM- Gut pond *Sarotherodon melanotheron*; GTLSM- Gut lagoon *Sarotherodon melanotheron.*

gill recorded 1.86±0.02×10^7 for *C. gariepinus,* 1.52±0.09×$10^{'}$ for *O. niloticus* and 1.63±0.05×$10^{'}$ for *S. melanotheron*. Buccal cavity recorded 1.37±0.07×10^6 for *C. gariepinus*, 1.29±0.21×10^6 for *O. niloticus* and 1.17±0.04×10^6 for *S. melanotheron*. Flesh recorded 6.03±0.35×10^4 for *C. gariepinus*, 2.67±0.03×10^4 for *O. niloticus* and 1.57±0.02×10^4 for *S. melanotheron*.

According to ICMF (1986) and Aitken et al. (1982) which say that any fish that have more than x10^6 bacteria count in one gram is not suitable for human consumption, but since gut and gill are always being removed and discarded, there is a tendency for safety, but the people should be encouraged not to consume them (gill and gut). Nevertheless, it is advisable (especially for those species from lagoon front) that good and effective processing treatment be employed such as washing, scraping of scales, removal of gill and gut (under a very hygienic condition), and proper cooking. These will help to reduce the microbial load on the flesh and muscle, thereby keeping the fish safe for human consumption.

It was also observed that even though there are no significance difference in the morphometric parameters of some of these fish species especially *O. niloticus* and *S. melanotheron* from Pond B, C and those of the Lagoon front, yet there was a high significance difference in their microbial populations. This suggests that the size or weight does not really determine the microbial load in any fish species, rather the nature of the fish environment and the extent of pollution around where fish lives. According to Cahill (1990), pathogens living in fish depend on the types and abundance of microorganism present in the water in which the fish lives. This can be inferred that the reason for higher population of bacteria in the lagoon front water and fishes as compared to the departmental pond was a function of pollution they were exposed to. Despite the fact that there was no significant difference in the morphometrics parameters of the fish species in the pond and thoe in the lagoon font yet the difference in their bacteria population was highly significant. The reason was simply because the departmental fishes were better catered for and their level of pollutions was being controlled through regular change of water. They were also not prone to discharge of domestic and industrial effluent unlike the lagoon front water.

The implication of this research finding to the fish shows that since the population of bacteria on the fishes from lagoon front was higher than the recommended limit, most of which are pathogenic; this may eventually lead to fish diseases and perhaps death. Also, the higher bacteria population in water can lead to increased biological oxygen demand and thus reduces the quantity of dissolve oxygen (DO) available for fish in the water; these reductions in DO will definitely impose stress on fish and give room for infection by pathogenic bacteria. Examples of fish bacterial diseases that could occur includes: enteric septicemia of catfish, motile aeromonas septicemia, mycobacteriosis, pseudominasis, vibriosis, and salmonellasis etc in a situation when man consumed a diseased fish there is a tendency to be infected by those pathogenic bacteria especially Zoonotic ones such as: *E. coli, Pseudomonas, Klebsiella, Edwardsiella, Vibrio Clostridium, Salmonella and Staphylococcus spp* (Stoskopf 1993).

Finally, effort should be made as much as possible to curtail the indiscriminate discharge of untreated sewage and industrial effluent into the lagoon as this will increase the microbial load in water and consequently inducing stress on fish and other aquatic organism present there. Good hygienic practices should be carried out when fish from the lagoon are purchased for consumption purposes. Bacteria in fish not properly cooked could be transferred to man as they establish themselves in the intestine particularly those that are pathogenic, leading to bacterial infections of various kinds. The good cultural and management practice in the Departmental ponds should be sustained and improved upon for higher productivity.

Conclusion

This study confirms the existence of pathogenic bacteria in the fish species analysed (*C. gariepinus, O. niloticus and S. melanotheron*) which are of public health significance. While the bacteria load recorded from the departmental ponds are still acceptable, being found within the acceptable limit of 10^6/g (ICMF, 1986; Aitken et al., 1982), their lagoon front counterparts were found to be densely populated, giving the TPC of the gut at 10^8, gill and mouth at 10^6 and 10^7 respectively. Although gill and gut are always being removed and discarded, yet it will require a careful handling to prevent contamination of other parts during processing, hence good hygiene and sanitation is very essential.

Moreover, bacteria isolated from the fish samples are a function of bacteria found in the lagoon which is influenced by industrial effluent, domestic and agricultural waste emptied into the lagoon. Findings have confirmed that fish can be infected with variety of microbial species especially bacteria, which is a function of bacteria found in their habitat (Olufemi, 1998).

Recommendations

Based on the findings during this study, the following recommendations are suggested.

(i) The Government, through Ministry of Environment, should enforce a regulation that will ensure that effluent or sewage are properly treated before being discharge into the lagoon, and this must be strictly adhere to by all industries and establishment concerned.
(ii) Government should make adequate provision for research grants to the relevant research institutes and institutions such as NIOMR, and Universities. This will give room for quality research work and thus help to improve the quality of water and fishes in our water bodies, for instance Lagos lagoon.
(iii) Fish should be properly processed before consumption in order to prevent the bacteria in the fish from infecting human.
(iv) Environmental education program and campaign should also be organized by the regulatory agency, this will help to put all hands on deck towards sustaining a good and save aquatic environment for our fisheries resources.

Conflict of Interest

There is no conflict of interest on this research paper.

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