Pathogenic Bacteria Diversity in *Clarias gariepinus* and their Seasonal Variation among Commercial Fish farms in Ota, Ogun State, Nigeria

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Abstract:

This study investigated the pathogenic bacteria diversity in African catfish (Clarias gariepinus), their seasonal variations, and public health significance. 135 samples were collected from the skin, gill and guts of 45 adult catfish from three major fish farms in Ota. Microbiological analysis was conducted on them during rainy, harmattan and dry seasons between April 2019 and February 2020 to determine the bacterial load and identify various pathogenic bacteria. Data collected were analyzed using Analysis of Variance. High bacteria diversity was observed across the three seasons and study areas. The total aerobic count of the gut at Royal Fish Farm ($1.24\times10^{7}\pm0.06^{c}$) was significantly higher ($P<0.05$) than that from the gill ($7.88\times10^{6}\pm1.22^{b}$), while at the gill, it was also higher than found in the skin ($6.08\times10^{5}\pm0.18^{a}$) during the rainy season. The same trend was observed during harmattan season ($1.93\times10^{7}\pm 0.28^{c}$, $1.45x10^{6} \pm0.06^{b}$, $8.44x10^{5} \pm 0.92^{a}$) and dry season ($2.14x10^{8} \pm 0.03^{c}$, $1.56x10^{7} \pm 0.07^{b}$, $1.45 x10^{6} \pm 0.03^{a}$). A similar trend was also observed at Farm 360 and Oluwadare Farm. Given the high contamination of bacteria pathogens recorded in this study, which exceeded the recommended limit of $<5.0x10^{5}$ colony forming unit by the International Commission for Microbiological Specification for Food, these fish can pose a high risk to human health if consumed. Given this, it is hereby recommended that the government should strictly enforce the environmental protection law to prevent indiscriminate disposal of industrial effluents and to keep the water body safe for aquatic lives and agricultural use.

Keywords: **Aquaculture, Bacteria Pathogens, High contaminations, Microbiological analysis, Public health significance.**

1. Introduction

Over the past decades, aquaculture has contributed significantly to global food production [1] [2]. Fish production (both capture and culture fisheries) generally contributes to one-fifth of all animal protein in the human diet [3]. Studies also showed that fish accounted for about 17 per cent of animal protein and 7 per cent of all proteins consumed by the global population [4]. Fish and fishery products provide an average of about 34 calories per capita per day; nevertheless, their daily contribution can exceed 130 calories per capita in countries such as Iceland, Norway, Japan, Korea, and several other Island states where alternative protein foods are scarce, and preference for fish has been developed and sustained [5]. In Nigeria, fish consumption accounts for over 40 % of the protein sources consumed daily [6]. Beyond being an energy source, the dietary contribution of fish is significant in terms of high quality and ease of digestion compared to other animal proteins [3] [4]. Fish is rich in protein, vitamins, and n-3 polyunsaturated fatty acids, which are very important, especially concerning nutritional health [7]. Fish also offers robust foreign exchange earnings because of its higher nutritional and health maintenance advantages, such as low cholesterol levels and the presence of essential amino acids above other animal protein sources [8] [9] [10]. However, pathogenic bacteria in fish can limit its productivity and render it a public health risk if necessary control measures are not implemented. Its safe consumption requires proper pond management practices and adequate sanitary conditions from harvest through processing to consumption. Consumption of contaminated fish can cause disease or infection based on the pathogenicity of the bacteria involved. Groups of gram-negative bacteria that can cause diseases in fish include *Salmonella* spp, *Escherichia coli*, *Vibrio* spp, *Enterobacter* spp, *Shigella* spp, *Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp, *Aeromonas* spp, *Flavobacterium spp*, *Serratia* spp, *and Citrobacter* spp. The gram-positive ones which are less prevalent include *Staphylococcus* spp, *faecal Streptococcus*, *Micrococcus* spp, *Bacillus* spp, and *Listeria* spp [11] [12].

Moreover, some bacteria are opportunistic, while others are obligatory [13]. Although only a few infectious agents in fish can infect humans, in a situation where a high population of pathogens are involved, it can be fatal if care is not taken [10]. Therefore, consuming unprocessed or insufficiently processed fish can pose a severe risk to human health [8] [14]. This study

is imperative because effluents from so many industries, hospitals, and homes in Ota, being a highly industrial and densely populated city, are discharged into the water body, where fish farmers channeled water into their earthen ponds, and this could pose a high risk to both fish and public health. Hence, it is necessary to investigate and ascertain the microbial status of the pond water and fish among some commercial fish farms in Ota.

Therefore, this study aims to determine the pathogenic bacteria diversities, their seasonal variation and public health significance among three commercial fish farms in Ota.

Samples collected were analyzed according to the basic concept of bacterial isolation procedures [15] [16], selective media were used in order to facilitate easy isolation and detection of the pathogens present in fish and water [17] while general purpose medium was used to determine the total aerobic count of the bacteria and their diversity. Serial dilution technique was also use to scale down the bacterial population for easy colony count on agar plate, and then back track to the unknown concentration using dilution factor. This helps to avoid too many bacteria in the plate which could be very difficult to count [18].

MATERIALS AND METHODS

Study Areas and Fish Sample Collection

Fish samples were collected from three reputable farms in Ado-Odo/Ota Local Government Area, Ota, Ogun State. These include the Royal fish farm, Ewupe, which is located at Latitude 6° 42ᴵN and Longitude 3°11ᴵE; Oluwadare fish farm, Arobieye, which lies at Latitude 6° 39ᴵN and Longitude 3° 8ᴵE, and Farm 360, Alagbon area, which also lies within Latitude 6° 41ᴵN and Longitude 3° 0ᴵE (Fig. 1).

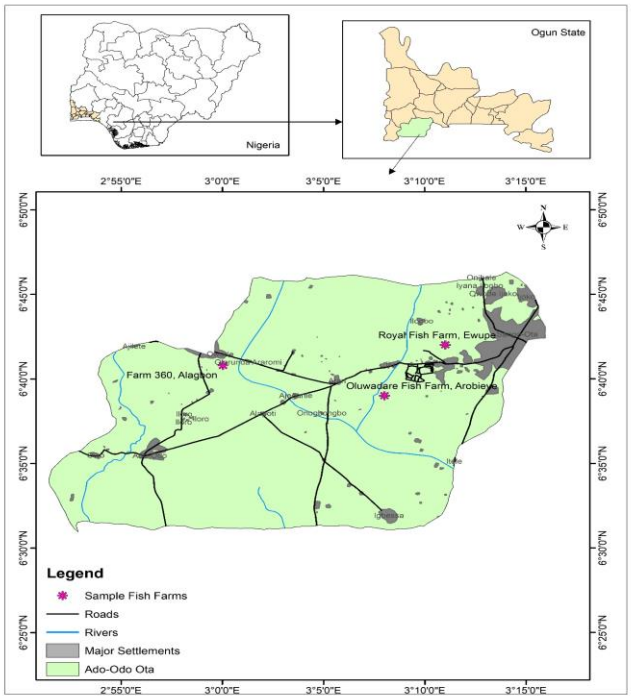

Fig. 1: Map of Ado-Odo/Ota Local Government Area, Ogun State, showing the study areas. Source: Olugbojo [19].

The fish samples were transported in sterile polythene bags, filled with pond water from each study area to the Microbiology Laboratory, Bells University of Technology, Ota. At the same time, sterile sample bottles were also used to collect water samples from each study area during rainy, harmattan and dry seasons for microbiological analysis.

Preparation of Serial Dilutions

90 ml of distilled water were dispensed into 100 ml conical flasks as diluents for each sample. The diluents were autoclaved at 121°C for 15 min. Nutrient Agar, MacConkey Agar, and Mannitol Salt Agar were also autoclaved along with the diluents. At the same time, *Salmonella Shigella* Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS) were brought to a boil on a hot plate according to the manufacturer's instructions.

Fish samples were taken aseptically, using sterile dissecting instruments, from the flesh, gill and gut. 10 g was weighed, pounded into pieces with sterile mortar and pestle, properly mixed, and added to 90 ml of sterile distilled water in each conical flask over a Bunsen burner flame. They were thoroughly mixed to make a ten-fold serial dilution 10^{-1}. Additional ten-fold serial dilutions were prepared using a sterile pipette from 10^{-1} to 10^{-5}. However, more dilutions till 10^{-9} were prepared and used, where necessary, for easy colony count. Each of the conical flasks was labelled sequentially. Likewise, for water samples, 10 ml was aseptically taken from pond water already kept in a sterile sample bottle into 90 ml of sterile distilled water in the conical flask to give dilution 10^{1}. Further, ten-fold serial dilutions were also prepared using a sterile pipette, from 10^{-1} to 10^{-5}, as done for fish samples, while the raw water sample remained 10^{0} [18] [20].

Inoculation

One ml (1 ml) of each inoculum was pipetted into sterile Petri dishes in two replicates and labelled sequentially. Using the pour plate technique, about 15 ml of sterile molten agar, cooled to about 45°C, were 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 were allowed to solidify. Some plates were also prepared as a control (containing no inoculum) to check the sterility of diluents, glass wares, and agar medium. The possibility of air contamination was also assessed using control plates. All prepared Plates were incubated in an inverted position at 37°C for 18-24 h.

With this procedure, Total Plate Count using Nutrient Agar, Total Coliform Count using MacConkey Agar, *Staphylococcus* spp counts using Mannitol Salt Agar, *Vibrio* spp counts using TCBS Agar, *Salmonella* spp and *Shigella* spp counts using *Salmonella Shigella* Agar were determined. Other bacteria which were present in the samples were also identified through further analytical procedures (Biochemical tests) as described by Fawole and Oso [15].

Bacterial count, colonial and morphological characteristics.

After incubation, all colonies on each Petri dish were counted and recorded with the aid of a Colony Counter. The colony-forming units per ml (water samples) and per gram (fish samples) were determined using the American Public

Health Association method (APHA) [21]. The bacterial count was multiplied by the dilution factor, divided by the volume of the inoculums, and expressed in a standard form. The Colony Counter was also used to examine and record the colonial characteristics, including colour, edge, shape, and elevation. Each distinct colony was sub-cultured into a freshly prepared Nutrient Agar for purification and then subjected to Gram stain to determine their reaction and cellular morphology [15].

Biochemical tests

Biochemical tests were carried out to identify the bacteria isolates further and then differentiate them from one another, especially the closely related species. Each organism on different culture media plates was sub-cultured on Nutrient Agar to obtain a discrete colony and pure culture. The following biochemical tests were carried out according to the standard procedures: Catalase test, coagulase test, citrate utilization test, oxidase test, sulphite and indole production test, motility test, urease test, sugar fermentation test (Glucose, Sucrose and Lactose), methyl red and voges-proskauer tests. [22] [23] [24] [25]

Analysis of Water Quality parameters

Pond water quality parameters such as Temperature, pH, Conductivity and Dissolved Oxygen (DO) were determined using a pH meter (Hanna instrument), Conductivity meter (Hanna instrument H186303), and Dissolved oxygen meter with an in-built thermometer (Smart Sensor AR 8210 instrument). All the instruments used were calibrated according to the manufacturer's instructions. Water quality parameters were analyzed during the rainy, harmattan and dry seasons. Temperature and Dissolved oxygen were measured simultaneously since the dissolved oxygen meter has an in-built Thermometer. The Dissolved oxygen meter was calibrated using zero DO solution (HI 7040), which was supplied along with the D.O meter by immersing the DO meter electrode into it. The calibration displayed 0.0 % DO. The electrode was rinsed using deionized water and cleaned with soft tissue paper. It was then immersed in the pond water to determine its actual DO. The value displayed on the screen was recorded when it became stable. This was conducted in two replicates, and the average was calculated. The water temperature shown on the screen and the dissolved oxygen values were also recorded in both trials. The average values were calculated and recorded. pH of the pond water was also determined in situ. The pH meter was calibrated using buffers (7.0 and 4.0 pH). After calibrations, the pH electrode was dipped directly inside the pond water, and the value displayed on the screen was recorded when it became stable. This was also conducted in two replicates, and the average was calculated. Electrical conductivity was determined ex-situ. A clean sample bottle was used to collect water samples from the pond and were taken to the laboratory. Deionized water was used to calibrate the conductivity meter. The conductivity meter electrode was immersed inside the pond water in the sample bottle. The values displayed on the screen were recorded when it became stable. This was conducted in two replicates, and the average was calculated [26] [27].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) and 10 Microsoft Excel spreadsheets. Means were separated using the Duncan multiple range test (DMRT) ($P<0.05$).

RESULTS

Bacteria detected in fish and water samples from Royal Fish Farm, Farm 360, and Oluwadare Farm during Rainy, Harmattan and Dry seasons

Generally, 554 bacteria isolates belonging to 15 different genera and 19 species were isolated in fish and water samples across the three study areas. 188 isolates were from Royal Fish Farm, 185 were from Farm 360, and 181 were from Oluwadare Farm, respectively (Tables 1, 2 and 3).

Bacteria groups such as Staphylococcus, Salmonella, Shigella, Vibrio, Coliform, and total aerobic counts were initially isolated to determine their population in the water and fish samples. However, other bacteria were also identified through biochemical tests. Tables 1, 2 and 3 depicted their variation in different study areas and seasons (Seasonal and study area variations), showing the specific season and area where each bacterium was found. These bacteria include *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Salmonella typhi, Shigella* spp*, Enterobacter aerogenes, Pseudomonas aeruginosa, Streptococcus faecalis, Klebsiella* spp*, Serratia* spp*, Proteus mirabilis, Micrococcus* spp*, Vibrio cholera, Aeromonas* spp*, Citrobacter* spp*, and Bacillus subtilis.*

Bacterial load of fish samples during Rainy, Harmattan, and Dry seasons in Royal Fish Farm

The bacterial load of fish samples in different body parts varied from skin to gill and gut. The population in the skin is lesser than that of the gill, while the population in the gill is also lesser than that in the gut (Skin<gill<gut) during the rainy season in the Royal Fish Farm (Table 4). This was observed in their Total plate count (TPC), Total coliform count (TCC), *Salmonella* spp*, Shigella* spp*, Vibrio* spp *and Staphylococcus* spp count showing significant differences along the rows ($P<0.05$), except for *Salmonella* spp (in the skin and gill only). In the harmattan season, the population also varies, as described in the rainy season. There were significant differences in the bacteria load along the rows on each bacteria type analyzed ($P<0.05$), except the total plate count, which showed no significant difference ($P>0.05$) in the skin and gill. The bacteria population during the dry season follows the same trend and varies as seen in the previous seasons; there were also significant differences along the rows ($P<0.05$), except for total coliform count and *Staphylococcus* spp count for skin and gill, respectively ($P>0.05$).

Bacterial load of fish samples isolated during Rainy, Harmattan, and Dry seasons in Farm 360.

The result obtained on the bacterial load of fish body parts (skin, gill, and gut) analyzed in Farm 360 (Table 5) is similar to what was obtained in Table 4 (Royal Fish Farm). The result also varies from skin to gill and gut increasingly (Skin<gill<gut) during the rainy, harmattan and dry season, as depicted in their Total plate count (TPC), Total coliform count (TCC), *Salmonella* spp*, Shigella* spp*, Vibrio* spp and *Staphylococcus* spp counts. There is no significant difference (P>0.05) in the bacteria load obtained in the skin and gill samples in all parameters (along each row) except the gut, which depicts a marked difference (P<0.05). TPC and *Staphylococcus* spp counts in the skin and gill during the harmattan season show no significant difference (P>0.05). In contrast, TCC, *Salmonella* spp*, Shigella* spp, and *Vibrio* spp counts differed significantly (P<0.05). The bacterial load in the gut sample (during harmattan) for all parameters along the row were highly significant (P<0.05). Also, the Salmonella spp and Staphylococcus spp populations on the analyzed skin and gill samples show no significant difference along the rows (P>0.05). In contrast, TPC and TCC of *Shigella* spp with *Vibrio* spp show considerable differences (P<0.05). The bacteria load on the gut sample for all bacteria analyzed showed a high significance difference along each row (P<0.05) compared to skin and gill samples.

TABLE 1
Bacteria detected in fish and water samples from Royal Fish Farm (Rainy, Harmattan and Dry season)

	Rainy season				Harmattan season				Dry season			
Types of bacteria	Skin	Gill	Gut	water	skin	Gill	Gut	water	Skin	Gill	Gut	water
Salmonella typhi	DE	ND	DE	DE	ND	ND	DE	DE	DE	ND	DE	DE
Salmonella typhimurium	ND	DE	DE	DE	DE	ND	DE	DE	ND	ND	DE	DE
Salmonella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Shigella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Escherichia coli	DE	ND	DE	DE	ND	ND	DE	DE	DE	ND	DE	DE
Enterobacter aerogene	ND	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
*Streptococcus fa*ecalis	DE	ND	DE	DE	DE	DE	DE	DE	ND	ND	DE	DE
Klebsiella spp	ND	ND	DE	DE	ND	ND	DE	DE	ND	DE	DE	DE
Serratia spp	ND	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
Proteus mirabilis	ND	DE	DE	DE	ND	DE	DE	DE	ND	ND	DE	DE
Staphylococcus aureus	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Staphylococcus spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Micrococcus spp	DE	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE
Vibrio cholera	DE	ND	DE	DE	DE	ND	DE	DE	DE	DE	DE	DE
Vibrio spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Citrobacter spp	DE	ND	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE
Aeromonas spp	ND	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
Pseudomonas aeruginosa	ND	DE	DE	DE	ND	DE	DE	DE	DE	DE	DE	DE
Bacillus subtilis	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE

Legend: DE – Detected, ND – Not Detected. Source: Olugbojo [19]

Bacterial load of fish samples isolated during Rainy, Harmattan, and Dry seasons in Oluwadare Farm

The bacterial load of the fish samples in the Oluwadare fish farm is depicted in Table 6. The bacterial load in different body parts varied from skin to gill and gut (Skin<gill<gut) during rainy, harmattan, and dry seasons. This could be observed in each fish sample's total plate count (TPC), total coliform count (TCC), Salmonella spp, Shigella spp, Vibrio spp and Staphylococcus spp count. There is no significant difference (P>0.05) in the TCC and *Staphylococcus* spp *count* in skin and gill samples during the rainy season. Still, the difference is substantial for TPC, Salmonella spp, Shigella spp and Vibrio spp (P<0.05). Moreover, the difference in the population of the gut samples when compared with skin and gill (along each row) for all the parameters is highly significant (P<0.05). The bacterial load during the harmattan season is similar to the rainy season. There was a considerable difference (P<0.05) in the TPC, *Salmonella* spp*, Shigella* spp and *Vibrio* spp counts on the skin and gill except TCC and *Staphylococcus* spp *count* (P>0.05). The TCC and Staphylococcus spp counts on the skin and gill during the dry season showed no significant difference (P>0.05), while TPC, *Salmonella* spp*, Shigella* spp*,* and *Vibrio* spp showed a high significant difference (P<0.05). Generally, there were significant differences when comparing the gut's bacterial load with skin and gill (P<0.05).

In addition, Tables 4-6 reveal a marked difference (P<0.05) in the bacterial load of the gut compared with gill and skin in each of the seasons and across the study areas. Also, the difference in the bacteria load of fish samples (skin, gill, and gut) during the dry season in each study area with those of the rainy and harmattan seasons is highly significant (P<0.05).

Bacterial load of water samples during Rainy, Harmattan and Dry seasons from Royal Fish Farm, Farm 360 and Oluwadare Fish Farm.

The bacterial load of water samples (Table 7) shows that the population was highest during the dry season in all the study areas and across the parameters (TPC, TCC, *Salmonella* spp, *Shigella* spp, *Vibrio* spp and *Staphylococcus* spp) compared with rainy and harmattan seasons.

TABLE 2
Bacteria detected in fish and water samples from farm 360 (Rainy, Harmattan and Dry seasons)

	Rainy Season				Harmattan				Dry Season			
Types of bacteria	Skin	Gill	Gut	water	Skin	Gill	Gut	water	Skin	Gill	Gut	water
Salmonella typhi	DE	ND	DE	DE	ND	DE	DE	DE	DE	DE	DE	DE
S. typhimurium	DE	ND	DE	DE	DE	ND	DE	DE	ND	DE	DE	DE
Salmonella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Shigella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Escherichia coli	DE	ND	DE	DE	ND	ND	DE	DE	ND	DE	DE	DE
E. aerogene	DE	ND	DE	DE	ND	ND	DE	DE	DE	ND	DE	DE
S.. faecalis	DE	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE
Klebsiella spp	DE	ND	DE	DE	DE	ND	DE	DE	ND	ND	DE	DE
Serratia spp	DE	ND	DE	DE	ND	DE	DE	DE	DE	ND	DE	DE
Proteus mirabilis	DE	ND	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
Staphylococcus aureus	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Staphylococcus spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Micrococcus spp	ND	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE
Vibrio cholera	DE	ND	DE	DE	DE	ND	DE	DE	ND	ND	DE	DE
Vibrio spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Citrobacter spp	ND	DE	DE	DE	ND	ND	DE	DE	ND	DE	DE	DE
Aeromonas spp	ND	ND	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
P. aeruginosa	DE	DE	DE	DE	ND	DE	DE	DE	ND	ND	DE	DE
Bacillus subtilis	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE

Legend: DE – Detected, ND – Not Detected
Source: Olugbojo [19]

For Total Plate Count (TPC): Farm 360 > Royal Fish Farm > Oluwadare Farm. But, in harmattan and rainy seasons, lower populations were recorded as follows: Royal Fish Farm (Harmattan) < Oluwadare Farm (Rain and Harmattan) < Farm 360 (Harmattan) < Royal Fish Farm (Rain) < Farm 360 (Rain).

For the Total coliform count (TCC): Oluwadare Farm > Royal Fish Fam > Farm 360. At the same time, the lower population were recorded in the following order: Farm 360 < Oluwadare Farm (Rain and Harmattan) < Farm 360 (Harmattan) < Royal Fish Farm (Rain and Harmattan).

For *Salmonella* spp: Farm 360 > Royal Fish Farm > Oluwadare Farm, while the lesser were observed in the following order: Oluwadare Farm (Harmattan) < Royal Fish Farm (Rain) < Farm 360 (Rain)

For *Shigella* spp: Farm 360 > Oluwadare > Royal Fish Farm while the lesser were found as follows: Farm 360 (Rain) < Royal Fish Farm (Harmattan)< Oluwadare Farm (Rain and Harmattan).

For *Vibrio* sp: Farm 360 > Royal Fish Farm > Oluwadare Farm while the lesser population were observed as follows: Oluwadare Farm (Harmattan)< Farm 360 (Rain)< Royal Fish Farm (Rain)

For *Staphylococcus* spp: Farm 360 > Royal Fish Farm > Oluwadare Farm while the lower population were found in the following order: Royal Fish Farm (Rain and Harmattan) < Farm 360 < (Rain and Harmattan) < Oluwadare Farm (Rain and Harmattan).

TABLE 3
Bacteria detected in fish and water samples from Oluwadare farms (Rainy, Harmattan and Dry season)

	Rainy				Harmattan				Dry Season			
Types of bacteria	Skin	Gill	Gut	Water	Skin	Gill	Gut	Water	Skin	Gill	Gut	Water
Salmonella typhi	DE	ND	DE	DE	ND	ND	DE	DE	ND	DE	DE	DE
S. typhimurium	ND	ND	DE	DE	ND	DE	DE	DE	ND	ND	DE	DE
Salmonella spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	ND	DE	DE
Shigella spp	DE	DE	DE	DE	DE	DE	DE	DE	ND	ND	DE	DE
Escherichia coli	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
E. aerogene	ND	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE
S. faecalis	ND	DE	DE	DE	ND	ND	DE	DE	DE	ND	DE	DE
Klebsiella spp	DE	ND	DE	DE	ND	DE	DE	DE	DE	DE	DE	DE
Serratia spp	DE	ND	DE	DE	ND	ND	DE	DE	ND	ND	DE	DE
Proteus mirabilis	DE	ND	DE	DE	DE	ND	DE	DE	ND	DE	DE	DE
S. aureus	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Staphylococcus spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Micrococcus spp	DE	ND	DE	DE	ND	DE	DE	DE	DE	DE	DE	DE
Vibrio cholera	DE	ND	DE	DE	DE	ND	DE	DE	ND	ND	DE	DE
Vibrio spp	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE
Citrobacter spp	DE	ND	DE	DE	ND	ND	DE	DE	DE	ND	DE	DE
Aeromonas spp	ND	ND	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
P. aeruginosa	DE	DE	DE	DE	ND	DE	DE	DE	ND	DE	DE	DE
Bacillus subtilis	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE	DE

Legend: De- Detected, N.D – Not Detected
Source: Olugbojo [19]

Generally, there is no significant difference (P>0.05) in the values of the TPC and TCC during the rainy and harmattan season across the three study areas except among *Salmonella* spp, *Shigella* spp, *Vibrio* spp, *Staphylococcus* spp whose difference are significant across the study area (P<0.05). For the dry season, the difference in the bacterial load of water (for all the parameters) is highly significant across the three study areas (P<0.05).

Colonial, Morphological and Biochemical characteristics of bacteria isolated in Clarias gariepinus from Royal Fish Farm, Farm 360, and Oluwadare farm

Table 8 depicted the colonial, morphological and biochemical characteristics of the bacteria isolated from the fish samples. Moreover, the table represents the general results of all the bacteria isolated across the three study areas and seasons in contrast to Table 1-3 which described the specific season and study area where each bacterium was found. Colonial characteristics such as pigmentation, edges, and elevation were determined according to Fawole and Oso [15].

For pigmentation, the following were observed: Colourless, colourless with a black centre, pink, cream, blue, green, yellow, and pale white colonies. Regular, undulate, irregular, rough and serrated colonies were also observed for edges. Convex, low convex, raised, and flat colonies were observed for elevation. Morphological characteristics of the bacteria cells also give the following results: cocci, bacilli, cocci in chain, irregular clusters, and curved rods. Biochemical characteristics were also determine. This includes the Catalase test, Coagulase Test, Citrate utilisation test, Urease test, indole test, oxidase test, Sulphite production test, motility test, methyl red test, Voges proskauer test, and sugar fermentation test (Glucose, Sucrose and lactose). [22] [23] [24] [25]. Probable bacteria that were identified include *Salmonella typhimurium*, *Salmonella typhi*, *shigella* spp, *E. coli*, *Staphylococcus aureus*, *Micrococcus* spp, *Vibrio cholera*, *Citrobacter* spp, *Aeromonas* spp, *Pseudomonas aeruginosa*, *Feacal streptococcus*, and *Bacillus subtilis*.

season in Royal Fish Farm and dry season in farm 360 (6.94±0.01b and 6.93±0.01b) (P>0.05). Dissolved oxygen (D.O) values measured in each of the study areas and seasons (rainy, harmattan and dry seasons) ranged from 5.57-6.91 mg/L. The D.O values show a significant difference (P<0.05) except those of the dry seasons in Farm 360 and Oluwadare Fish Farm (6.88±0.01b and 6.86±0.01b) (P>0.05).

DISCUSSION

Bacterial load of Fish samples in Royal Fish Farm, Farm 360 and Oluwadare Farm during Rainy, Harmattan and Dry seasons

Bacterial load in the gut across the three study areas and seasons

Gut recorded the highest population of bacteria for each of the parameters, most especially during the dry season. This result shows that among the bacteria found in the gut, TPC carries the highest bacterial population at 10^8 for all dry seasons and 10^7 for the harmattan and rainy seasons, which is expected to be the total aerobic count in the gut, followed by the TCC at 10^7 for all dry seasons, and 10^6 for both harmattan and rainy seasons. Salmonella spp and Vibrio spp were at 10^6 for all dry seasons and 10^5 for both harmattan and rainy seasons. Shigella spp and Staphylococcus spp were at 10^5 for all dry seasons and 10^4 for both harmattan and rainy seasons. This result corroborates the previous reports [28] [29], which stated that season affects the microbial population in fish, especially during the dry season when water evaporation is higher than condensation. Also, Akinyemi and Buoro [30] reported high Proteus spp and Streptococcus pyogenes infection in fish liver and gut due to their heavy presence in

TABLE 4
Bacterial load of fish samples in Royal Fish Farm during Rainy, Harmattan and Dry seasons (cfu/g ±SD)

	Rainy season			Harmattan season			Dry season		
Type of Bacteria	Skin	Gill	Gut	Skin	Gill	Gut	Skin	Gill	Gut
Total plate count	6.08 x10^5 ± 0.18^a	7.88x1$0^6$ ± 1.22^b	1.24x10^7 ± 0.06^c	8.44 x10^5± 0.92^a	1.45x10^6 ± 0.06^b	1.93x10^7 ± 0.28^c	1.45x1$0^6$ ± 0.03^a	1.56x1$0^7$ ± 0.07^b	2.14x10^8 ± 0.03^c
Total coliform count	2.82 x10^4± 0.01^a	2.67x1$0^5$ ± 0.33^b	2.66 x10^6± 0.20^c	8.20 x10^4± 0.61^a	2.57x10^5±0.07^b	2.91x10^6 ± 0.34^c	2.10x1$0^5$ ± 0.11^a	2.74x1$0^6$ ± 0.35^a	4.24x10^7 ± 0.41^b
Salmonella spp	8.68x10^3 ± 0.30^a	3.75 x10^4± 0.23^a	7.88 x10^5± 0.54^b	1.38x1$0^3$ ± 0.04^a	3.14x10^4 ± 0.04^b	7.62 x10^5± 0.23^c	2.65x1$0^4$ ± 0.07^a	1.97 x10^5± 0.11^b	8.54x10^6 ± 0.06^c
Shigella spp	3.6x10^2 ± 0.6^a	9.30 x10^3± 0.15^b	8.79x10^4 ± 0.56^c	2.4 x10^2± 0.5^a	2.50x10^3 ± 0.07^b	3.78 x10^4± 0.05^c	1.34 x10^3± 0.05^a	2.04x1$0^4$ ± 0.04^b	2.40x10^5 ± 0.06^c
Vibrio spp	2.40 x10^3± 0.25^a	2.88 x10^4± 0.05^b	6.69x10^5 ± 0.32^c	1.60 x10^3± 0.14^a	2.07x10^4 ± 0.07^b	2.78x10^5 ± 0.05^c	2.10 x10^4± 0.04^a	1.06x1$0^5$ ± 0.03^b	3.02x10^6 ± 0.03^c
Staphylococcus spp	3.2x10^2±1.1^a	7.52x1$0^3$ ± 0.55^b	3.28x10^4 ± 0.25^c	8.0x10^2 ± 1.40^a	8.02 x10^3± 0.08^b	5.52x10^4 ± 0.11^c	1.06 x10^3± 0.05^a	1.18x1$0^4$ ± 0.05^a	3.50x10^5 ± 0.71^b

Source: Olugbojo [19]

Footnote: Valu**e =** Mean ± SD

Mean ±S. D with a superscript of the same alphabet along the rows shows no significant difference (P>0.05)

Mean ± S.D. with superscripts of the different alphabet along the rows shows that there was a significant difference (P<0.05)

Water quality parameters of Royal Fish Farm, Farm 360, and Oluwadare Farm

The results of the water quality parameters of Royal Fish Farm, Farm 360, and Oluwadare Farm are shown in Table 9. Water temperature ranged between 23.73 and 30.00 0C. Statistical analysis revealed a significant difference in water temperatures in different seasons and study areas (P<0.05). Water conductivity also ranged between 96.50 – 147.50 µS/cm. There is a significant difference in the Conductivity values across the three seasons and study areas. The pH value ranged between 6.15-6.94. The pH values depict a considerable difference (P<0.05) except during harmattan

water bodies from where they enter the fish and accumulate in the liver and gut. They also observed that bacteria were more established in the gut than other body parts, especially pathogenic Salmonella spp.

Bacteria load in the gill across the three study areas and seasons

Gill of *Clarias gariepinus* recorded a higher bacteria population apart from the gut during the rainy, harmattan and dry seasons for each parameter across the three study areas. The results showed that the total plate count gave the highest microbial load, especially during the dry season (10^7), while the rainy and harmattan seasons gave a total plate count of 10^6. The total coliform count was recorded at 10^6 for the dry season and 10^5 for the rainy and harmattan seasons. Salmonella spp and Vibrio spp counts gave the bacteria load at 10^5 for the dry season and 10^4 for both rainy and harmattan seasons. In contrast, Shigella and Staphylococcus spp counts were recorded at 10^3 for the dry season and 10^2 for the rainy and harmattan seasons. This result is similar to the previous findings [30], where more bacteria population was recorded in the gill than in the skin and buccal cavity.

Bacterial load in the skin across the three study areas and seasons

TPC analysis of the skin samples showed the highest bacterial load during the dry season (10^6). TPC analysis during the rainy and harmattan seasons depicted 10^5, followed by TCC at 10^5 for the dry season and 10^4 for the rainy and harmattan seasons. *Salmonella* and *Vibrio sp* were recorded at 10^4 for the dry season and 10^3 for the rainy and harmattan seasons. At the same time, *Shigella* and *Staphylococcus spp* gave their bacteria load at 10^3 for the dry season and 10^2 for the rainy and harmattan seasons. The result found in the skin samples shows that skin has the least bacteria load among the three body parts analyzed. This result is also in tandem with the other researchers' finding [31], in which the bacteria population in the skin sample was less than that of the gut and gill.

The results so far showed that the fish experience more contamination during the dry season than the rainy and harmattan seasons in all the study areas. Moreover, the TPC, TCC, Salmonella spp and Vibrio spp counts of the gut analysis during the dry season depicted a higher bacterial load beyond the recommended limit of $<5.0x10^5$ cfu/g by the International Commission for Microbiological Specification for Food [32] which can be very hazardous if consumed. Also, the TPC of gill samples during rainy and harmattan seasons, TPC of skin and TCC of gill samples during the dry season recorded a higher bacterial contamination beyond the recommended limit of $<5.0x10^5$ cfu/g. In addition, Vibrio spp counts of gill and Staphylococcus spp counts of gut samples in Farm 360, with Shigella spp counts of gut samples in Oluwadare Farm during the dry season recorded a higher bacterial contamination beyond the recommended limit of $<5.0x10^5$ cfu/g [32].

TABLE 5
Bacterial load of fish samples in Farm 360 during Rainy, Harmattan and Dry seasons (cfu/g±SD)

	Rainy season			Harmattan season			Dry season		
Type of Bacteria	Skin	Gill	Gut	Skin	Gill	Gut	Skin	Gill	Gut
Total plate count	$2.58x10^5$ ± 0.40^a	$1.61x10^6$ ± 0.54^a	$2.84x10^7$ ± 0.24^b	$4.80x10^5$ ± 0.54^a	$1.46x10^6$ ± 0.25^a	$2.50x10^7$ ±0.24^b	$2.63x10^6$ ± 0.32^a	$8.30x10^7$ ±0.02^b	$5.42x10^8$ ±0.37^c
Total coliform count	$2.31x10^4$ ± 8.5^a	2.53 $x10^5$± 0.31^a	$2.60x10^6$ ± 0.23^b	$8.31x10^4$ ± 0.36^a	$8.04x10^5$ ± 0.80^b	1.23 $x10^6$± 0.13^c	$1.04x10^5$ ± 0.27^a	$1.89x10^6$ ± 0.15^b	$1.45x10^7$ ±0.16^c
Salmonella spp	$1.44x10^3$ ± 0.36^a	$1.99x10^4$ ± 0.58^a	$5.70x10^5$ ± 0.71^b	1.44 $x10^3$± 0.36^a	$7.70x10^4$ ± 0.63^b	$2.25x10^5$ ± 0.35^c	$3.42x10^4$± 0.51^a	2.46 $x10^5$± 0.43^a	$7.60x10^6$± 0.01^b
Shigella spp	$3.00x10^2$ ± 0.1^a	$2.50x10^3$ ± 0.30^a	$9.30x10^4$ ± 1.70^b	3.00 $x10^2$± 1.00^a	6.18 $x10^3$± 0.34^a	$4.29x10^4$ ± 0.91^b	4.20 $x10^3$± 0.86^a	7.23 $x10^4$±0.72^b	3.95 $x10^5$± 0.11^c
Vibrio spp	$7.00x10^3$ ± 0.86^a	$1.51x10^4$ ± 0.52^a	$2.74x10^5$ ± 0.18^b	$7.00x10^3$ ± 0.57^a	$2.87x10^4$ ± 0.45^b	6.56 $x10^5$±0.21^c	2.15 $x10^4$± 0.02^a	$9.34x10^5$ ± 0.45^b	$1.62x10^6$ ±0.170^c
Staphylococcus spp	$1.40x10^2$ ± 0.5^a	$1.12x10^3$ ± 0.08^a	$2.36x10^4$ ± 0.35^b	$6.20x10^2$ ± 0.8^a	$2.56x10^3$ ± 1.45^a	$4.10x10^4$ ± 0.04^b	$3.12x10^3$ ± 0.31^a	$3.03x10^4$ ± 0.40^a	6.46 $x10^5$± 0.49^b

Source: Olugbojo [19]
Footnote: Valu**e** = Mean ± SD
Mean ±S. D with a superscript of the same alphabet along the rows shows no significant difference (P>0.05)
Mean ± S.D. with superscripts of the different alphabet along the rows shows that there was a significant difference (P<0.05)

Bacterial load of pond water samples in Royal Fish farm, Farm 360, and Oluwadare Farm

A higher population of bacteria was observed during the dry season in each of the study areas than in rainy and harmattan seasons, especially the total plate count. This suggested the reason for recording a higher microbial population in fish body parts during the dry season compared with rainy and harmattan seasons. Taiwo et al. [31]. However, the microbial population did not exceed the recommended limit of $<5.0x10^5$ cfu [32] in all three seasons and study areas, including the total plate count, which was highest in Farm 360 during the dry season at $4.60x10^5$ cfu/ml

Water quality parameters of Royal Fish Farm, Farm 360, and Oluwadare Farm during Rainy Harmattan and Dry seasons

Water temperature ranged between 23.73 and 30.00°C. It falls within the recommended ranges of 25.00-32.00 0C for tropical fishes [33], except those values recorded during the Harmattan season in Royal Fish Farm, Farm 360, Oluwadare Farm, and rainy season in Royal Fish Farm. Water conductivity also ranged between 96.50±0.71b – 147.50±0.71a µS/cm. It falls within a typical Nigeria inland water conductivity range of 50-150 µS/cm [28]. The pH falls within the acceptable limit of 6.2-7.4 for fish growth [33].

Finally, the bacterial load of the skin, gill, and gut of *Clarias gariepinus* in each of the study areas and seasons depicted variations in all the microbiological parameters that were analyzed, especially during the dry season. The presence of *Salmonella* spp found in higher populations, especially in the gut, could either be attributed to the state of the fish feed because, most times, *Salmonella* spp pathogens gain entry into fish ponds through contaminated fish feed or when supplementary feeds such as chicken offal, fish gut, spoilt eggs are fed to fish which is the common practice among farmers in these study areas [37].

Incidence of *Vibrio* spp pathogens in fish ponds has been

TABLE 6
Bacterial load of fish samples in Oluwadare Farm during Rainy, Harmattan and Dry seasons (cfu/g ± SD)

	Rainy season			Harmattan season			Dry season		
Type of bacteria	Skin	Gill	Gut	Skin	Gill	Gut	Skin	Gill	Gut
Total plate count	$2.44x10^5$ ±0.41a	$2.30x10^6$± 0.59b	$2.07x10^7$ ±0.87c	$8.24x10^5$ ± 0.40a	$7.31x10^6$ ± 0.15b	$5.61x10^7$ ±0.36c	$1.26x10^6$ ± 0.03.(	$2.35x10^7$ ±0.28b	$2.74x10^8$ ±0.21c
Total coliform count	$1.90x10^4$ ± 0.48a	$1.87x10^5$± 0.49a	$1.68x10^6$ ± 0.38b	$1.62x10^4$ ± 0.06a	$1.07x10^5$ ± 0.02a	$1.42x10^6$ ± 0.04b	$3.47x10^5$ ±0.46a	$1.23x10^6$ ± 0.22a	$6.48x10^7$ ± 0.27b
Salmonella spp	3.02 $x10^3$± 0.32a	$2.62x10^4$ ±0.29b	$3.13x10^5$± 0.05c	1.38 $±10^3$ 0.10a	$1.47x10^4$ ± 0.05b	$1.55x10^5$ ± 0.05c	$3.14x10^5$ ± 0.20a	$2.48x10^5$ ± 0.35b	$1.64x10^6$ ± 0.09c
Shigella spp	$2.00x10^2$ ± 0.7a	$2.60x10^3$ ± 0.52b	$2.21x10^3$± 0.28c	$6.60x10^2$ ± 0.9a	$2.60x10^3$ ± 0.52b	$1.17x10^4$ ± 0.05c	$5.04x10^3$ ± 0.36a	$7.19x10^4$± 0.35b	$9.21x10^5$ ± 0.07c
Vibrio spp	$5.98x10^3$ ± 0.19a	$2.24x10^4$ ± 0.48b	$1.52x10^5$ ± 0.08c	$8.64x10^3$ ± 0.72a	2.24 $x10^4$± 0.48b	$2.77x10^5$± 0.13c	$2.43x10^4$ ± 0.12a	$2.48x10^5$ ± 0.13b	$2.12x10^6$ ± 0.18c
Staphylococcus spp	$2.80x10^2$± 0.40a	$7.80x10^3$ ± 0.83a	1.52 $x10^4$± 0.32b	$5.60x10^2$ ±1.1a	$5.76x10^3$± 0.65a	$2.55x10^4$± 0.25b	$1.62x10^3$ ± 0.81a	$2.42x10^4$ ± 0.36a	$2.65x10^5$ ± 0.42b

Source: Olugbojo [19]
Footnote: Valu**e =** Mean ± SD
Mean ±S. D with a superscript of the same alphabet along the rows shows no significant difference (P>0.05)
Mean ± S.D. with superscripts of the different alphabet along the rows shows that there was a significant difference (P<0.05)

The Dissolved Oxygen values measured in each of the study areas and seasons showed that the results are within the acceptable limit of 5-7 mg/L for fish growth, according to Tower [33] and Stickney [35]. Moreover, the dissolved oxygen values, which were found to be highest during the dry season against the usual expectation, could result from the current climate change trend (although all water quality testing was conducted in the morning and evening). This result was supported by Uzukwu et al. [36], who also recorded higher D.O. during the dry season.

reported due to effluent discharge from nearby industries, hospitals and homes to the river, from where they gain entry into the fish pond [38]. Earthen ponds are usually constructed close to the flowing river, from where water is channeled into the pond. Therefore, microbial contaminant can be received through untreated effluents from the surrounding industries [38, 39]. In some cases, it could be due to human faeces directly discharged into the river from where fish ponds receive water. *Shigella* spp contamination could be due to effluent from the nearby industries or from the fish feed [40].

TABLE 7
Bacterial load of water samples (in cfu/mL ± SD) from Royal Farm, Farm 360 and Oluwadare Farm

Type of	Royal Fish Farm			Farm 360			Oluwadare Farm		
bacteria	Rainy	Harmattan	Dry	Rainy	Harmattan	Dry	Rainy	Harmattan	Dry
TPC	$5.30x10^3$± 0.10^d	$2.01x10^3$± 0.01^d	3.20x10 $±0.10^b$	$8.06x10^3$± 0.01^d	$3.17x10^3$± 0.15^d	$4.60x10^5$± 0.10^a	$2.97x10^3$± 0.01^d	$2.87x10^3$± 0.06^d	$1.81x10^5$± 0.01^c
TCC	$4.00x10^2$± 0.10^d	$2.80x10^2$±0 $.01^d$	$2.73x10^4$ $±0.01^b$	$1.10x10^2$± 0.10^d	$1.60x10^2$± 0.10^d	$1.53x10^4$± 0.01^c	$1.40x10^2$± 0.01^d	$1.50x10^2$±0. 01^d	$6.58x10^4$±0 $.07^a$
Salmonella spp	$6.00x10^1$± 0.01^e	$8.00x10^1$±0 $.01^a$	$3.20x10^3$ $±0.10^b$	$5.00x10^1$± 0.01^{de}	$1.10x10^2$± 0.10^d	$6.10x10^3$± 0.10^a	$7.00x10^1$± 0.01^{de}	$3.00x10^1$±0. 01^{de}	$2.07x10^3$±0 $.01^c$
Shigella spp	$4.00x10^1$± 0.01^d	$1.50x10^1$±0 $.01^e$	$3.10x10^2$ $±0.01^c$	$1.10x10^1$± 0.01^e	$4.00x10^1$± 0.01^d	$7.20x10^2$± 0.02^a	$2.00x10^1$± 0.01^e	$2.00x10^1$±0. 01^e	$4.40x10^2$±0 $.01^b$
Vibrio spp	$7.00x10^1$± 0.01^f	$7.60x10^2$±0 $.10^d$	$3.40x10^3$ $±0.10^b$	$5.00x10^1$± 0.01^f	$1.20x10^2$± 0.10^f	$5.60x10^3$± 0.10^a	$4.60x10^2$± 3.50^e	$2.00x10^1$±0. 00^f	$1.12x10^3$±0 $.02^c$
Staphylococcus spp	$1.00x10^1$± 1.00^a	$1.50x10^1$±0 $.01^a$	$1.40x10^2$ $±0.10^b$	$1.60x10^1$± 0.01^a	$3.00x10^1$± 0.10^a	$4.80x10^2$± 0.10^a	$2.00x10^1$± 0.01^a	$2.80x10^1$±0. 01^a	$1.30x10^2$±0 $.10^a$

Source: Olugbojo [19]
Footnote: Valu**e =** Mean ± SD
Mean ±S. D with a superscript of the same alphabet along the rows shows no significant difference (P>0.05)
Mean ± S.D. with superscripts of the different alphabet along the rows shows that there was a significant difference (P<0.05)

In contrast, Staphylococcus spp contamination usually occurs during harvesting, especially when contaminated materials are in all three body parts, followed by the gill and the lowest in the skin. This is because the gut is like a "dung site" which

TABLE 8
Colonial, Morphological and Biochemical characteristics of bacteria isolated in *Clarias gariepinus* from Royal Fish Farm, Farm 360, and Oluwadare Farm

Organism Codes	Edges	Colour	Elevation	Shape	G. stain	Cat	Coa	Cit	Ox	Ur	H_2S	Ind	Mot	Lact	Glu	Suc	VP	MR	Probable bacteria
OHSKb	Regular	Colour-less with a Black center	Low convex	Rod/ bacillus	-	+	-	-	-	-	+	-	+	-	A	-	NA	NA	*S. typhimurium*
ORSKa	Regular	Colour-less with black center	Low convex	Rod	-	+	-	-	-	-	+	-	+	-	A	-	NA	NA	*S. Typhi*
RDSKa	Regular	Colour-less with a black center	Low convex	Rod	-	-	+	+	-	-	+	-	+	-	AG	-	NA	NA	*Salmonella sp*
RDSKb	Regular	Colour-less	Convex	Rod	-	+	-	-	-	-	-	-	-	-	A	-	NA	NA	*Shigella* sp
ODSKb	Undulate	Red/ Pink	Slightly raised	Rod	-	-	-	-	-	-	-	+	+	AG	AG	AG	-	+	*E. coli*
OHGTb	Serrated	Cream pink	Raised	Rod	-	+	-	+	-	-	-	-	+	AG	AG	AG	+	-	*Enterobacter aerogene*
ODGTb	Irregular	Blue green	Raised	Rod	-	+	-	+	+	+	-	-	-	-	A	-	NA	NA	*Pseudomonas aeruginosa*
AHGLa	Irregular	Grey	Raised	Cocci In short Chains	+	-	-	NA	NA	NA	NA	NA	-	AG	AG	AG	NA	NA	*Streptococcus faecalis*
OHSKc	Undulate	Cream	Slightly raised	Rod	-	-	+	+	-	+	-	-	-	AG	AG	AG	+	-	*Klebsiella* sp
ARGLc	Undulate	Red	Convex	Rod	-	+	-	-	-	-	-	-	+	A	A	-	+	-	*Serratia* sp
ADSKc	Undulate	Cream	Flat	Rod	-	+	-	+	-	+	+	+	+	-	AG	-	NA	NA	*Proteus mirabilis*
AHGLa	Irregular	Yellow	Raised	Cocci in clusters	+	+	+	NA	NA	-	-	-	-	A	A	A	NA	NA	S. *aureus*
ODGTc	Regular	Bright yellow	Convex	Cocci in irregular clusters	+	+	-	-	+	-	-	-	+	-	-	NA	NA	NA	*Micrococcus* sp
AHGLa	Regular	yellow	Raised	Curved Rod	-	+	-	+	+	NA	-	+	+	-	A	A	-	-	*Vibrio cholera*
ODGTb	Regular	Green	Raised	Curved Rod	-	+	-	+	+	NA	-	+	+	-	A	-	-	-	*Vibrio* sp
ARGLd	Regular	gray	Convex	Rod	-	+	-	+	-	-	-	+	+	AG	AG	-	-	+	*Citrobacter sp*
ODGTa	Regular	Pale white	Convex	Rod	-	+	-	+	+	+	+	+	+	AG	AG	AG	+	-	*Aeromonas sp* spp
ORSKe		slightly yellow	Flat	Rod	+	+	NA	+	+	-	+	-	+	-	+	A	A	-	*Bacillus subtilis*

Legend

H_2S- Hydrogen sulphite
Cat–catalase
Coa– coagulase
Cit– Citrate
Oxi– oxidase
Glu – Glucose
Suc– Sucrose
NA- Not applicable
AG- Acid and Gas
A - Acid

Lac- Lactose
VP – Voges Proskauer
MR– Methyl red
Ur-Urease RDSK1b
G. Stain– Gram's stain

DGTb- Oluwadare, Dry, gut, organism b
AHGla- Alagbon (F. 360), Hamattan, gill, organism a
OHSkc-Oluwadare, Hamattan, skin, organism c
ARGlc- Alagbon, Rain,Gill, organism c
ADSkc- Alagbon, Dry, Skin, organism c
ORSke- Oluwadare, Rain, Skin, Organism e
AHGla- Alagbon, Hamattan, Gill, organism a
RDSKa- Royal, Dry, skin, organism a
RDSkb- Royal, Dry, skin, organism b
ODSkb- Oluwadare, Dry, skin, organism b
OHGtb- Oluwadare, Hamattan, gut, organism b
ORSKa- Olwadare, rain, skin, organism a
OHSKb- oluwadare, hamattan, skin, organism b
ODGtc- Oluwadare, Dry, Gut, organism c
AHGla- Alagbon, Hamattan, Gill, organism a
ODGtb- Oluwadare, Dry, Gut, Organism b
ARGLd- Alagbon, Rain, Gill, Organism d
ODGta- Oluwadare, Dry, Gut, organism a

Source: Olugbojo [19]

used. It was also reported that they accumulate in the intestine and find their way into fish faeces, polluting pond water [12] [41]. Generally, microbial contamination occurs due to poor hygiene and sanitation standards during cultivation, feeding, sorting, harvesting, and processing [42].

Moreover, the gut carries the highest population of bacteria harbours most of the metabolic wastes and several undigested foods which attract high concentrations of bacteria [43] from where they are passed to the large intestine and then released as faeces. Gill also harbours much bacteria through its gill raker, which filters water that enters the fish body. The dirt the gill raker collects accumulates, and thus, microorganisms find

TABLE 9
Water quality parameters of Royal Fish Farm, Farm 360, and Oluwadare Farm

	Royal Fish Farm			Farm 360			Oluwadare Farm		
Parameters	Rainy	Harmattan	Dry	Rainy	Harmattan	Dry	Rainy	Harmattan	Dry
Temperature (^{0}C)	25.30±0.00^{g}	24.59±0.01^{h}	30.00±0.01^{a}	25.80±0.01^{e}	23.73±0.00^{f}	28.70±0.01^{c}	25.83±0.00^{d}	24.31±0.01^{i}	29.50±0.01^{b}
Conductivity (µS/cm)	96.50±0.71^{h}	125.00±0.00^{de}	147.50±0.71^{a}	114.50±0.70^{f}	123.50±0.71^{e}	136.50±0.71^{b}	100.50±0.70^{g}	125.50±0.71^{d}	130.50±0.70^{c}
pH	6.15±0.01^{h}	6.94±0.01^{b}	6.68±0.01^{e}	6.30±0.01^{g}	6.97±0.01^{a}	6.93±0.01^{b}	6.50±0.01^{f}	6.84±0.01^{d}	6.90±0.01^{c}
D.O (Mg/L)	5.93±0.00^{f}	6.51±0.01^{d}	6.91±0.02^{a}	5.57±0.01^{h}	6.68±0.01^{c}	6.88±0.01^{b}	5.86±0.01^{g}	6.20±0.01^{e}	6.86±0.01^{b}

Source: Olugbojo [19]
Footnote: Valu**e =** Mean ± SD
Mean ±S. D with a superscript of the same alphabet along the rows shows no significant difference (P>0.05)
Mean ± S.D. with superscripts of the different alphabet along the rows shows that there was a significant difference (P<0.05)

their way into it. The skin which carries the minor population of bacteria among the three-body parts could be because it is the closest part to the water body. With the water movement, there is a high possibility of washing off every micro-organism clinging on the fish body except where there is bruise or wound which can allow deep penetration into the fish body. There were higher populations of bacteria in the water samples during the dry season than in the rainy and harmattan seasons across the three study areas. This could be because, during the rainy season, there is a high level of dilution, which tends to reduce the population of bacteria in 1 ml of water, unlike dry and harmattan seasons in which no dilution occurs (due to lack of rainfall). Thus, bacteria keep multiplying, most especially during the dry season. The dry season also provides a suitable environment for bacteria to flourish than the rainy season. A higher population of bacteria in the fish body than in the water sample could be due to the continuous accumulation and multiplication of bacteria in the fish body, being a naturally suitable habitat for bacteria growth [44].

The physicochemical parameters of water support the growth of the fish in all study areas except in a few cases during the harmattan season, where there was a shortfall in temperature, coupled with higher Conductivity during both harmattan and dry season, which presupposes food decomposition by microbes (bacteria) which tend to increase the microbial load of water [34].

CONCLUSION

Based on the findings in this research, there are heavy bacterial contaminations in all the study areas, especially during the dry season. This shows that there is a need for urgent governmental intervention to prevent possible disease outbreaks, being among the foremost fish farms within the Ado-Odo/Ota local government area of Ogun state, Nigeria. To guide against this potential pandemic, it is recommended that fish gills and gut should not be consumed and must be adequately treated before being discarded. Thorough boiling is also recommended before consumption during fish processing. For fish farmers who use fish gills and gut to feed their pond fish, it is advisable to boil them properly before feeding them to fish to avoid possible bacterial contamination and incidence of fish diseases. Finally, regular change of pond water and proper treatment where necessary is highly recommended since water quality is a major determinant factor of fish health, and the public in general, when the fish are consumed. Above all, the Ministry of Environment and Federal Environmental Protection Agency should enforce environmental protection law which stated the manner by which effluents and wastes should be disposed.

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CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest in this research.

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