



# **Bacteriological Assessment of Fish and Pond Water in Fish Farms that Use Chicken Droppings as Feeds in Keffi, Nigeria**

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## **Abstract**

Fish farming or pisciculture involves commercial breeding of fish, most often for food, in fish tanks or artificial enclosures such as fish ponds. This study aimed at bacteriological assessment of fish and pond water in fish farms that use chicken droppings as feeds in Keffi. A total of forty-eight (48) samples, pond water thirty-six (36) and fish, twelve (12) were collected from four (4) different fish farms in Keffi. Bacteriological assessment of the pond water and fish samples were carried out using standard microbiology techniques. The highest total viable count from fish pond water samples  $29.03 \times 10^6 \pm 1.02$  was obtained from Pond A, while highest total viable count from fish samples  $12.12 \times 10^6 \pm 0.63$  was obtained from Pond B. the highest bacteria isolated from pond water was *E. coli* (38.8%) while *Salmonellaspp* was lowest bacteria isolated (19.4%). *E. coli* and *Klebsiellaspp* had the highest occurring bacteria from the fish skin 50.0% while *Citrobacterspp* was the lowest occurring bacteria of 25.0%. In hemolysin production for bacterial isolated from pond water out of 7 *Salmonella spp* 3(42.8%), of 8 *Citrobacterspp* 4(50.0%), out of 11 *Klebsiellaspp* isolated 5(45.5%) and out of 14 *E. coli* 3 (21.4 %). From the fish skin out of 4 *Salmonella spp* 2(50.0), 3 *Citrobacterspp* isolated 1(33.3), out of 6 *Klebsiellaspp* isolated 2 (33.3 %) and out of 14 *E. coli* 6 4 (66.6%) were hemolysin producers For the pond water samples, 71.4% of *Salmonellaspp*, 37.5% of *Citrobacterspp*, 54.5% of *Klebsiellaspp* and 57.1% of *E. coli* banded with Congo Red dye. Also, for the fish samples, 75.0% of *Salmonellaspp*, 66.6% of *Citrobacterspp*, 50.0% of *Klebsiellaspp* and 66.6% of *E. coli* banded with Congo red dye. It was observed in this study that different bacteria species isolated were pathogenic bacteria.

**Keywords:** fish pond, water, fish skin, Fish farm, congo Red dye, hemolysin production

## Introduction

Fish farming is currently a significant sector in global food production in Nigeria and the World. It is also a vital source of food and categorically a source of animal protein essential for both human and livestock nutrition. The farming of aquatic organisms (for example fish) is known as aquaculture. Fish farming or pisciculture involves commercial breeding of fish, most often for food, in fish tanks or artificial enclosures such as fish ponds. It is a particular type of aquaculture, which is the controlled cultivation and harvesting of aquatic animals such as fish, crustaceans, molluscs and so on, in natural or pseudo-natural environments (Zheng, 2022). A pond is referred to as a man-made or natural water body which is between 1m<sup>2</sup> and 20,000m<sup>2</sup> in area which holds water for at least four months of the year or all year around depending on geographic locations (Gogoi and Sharma, 2013). Fish ponds are either natural or manmade aquatic ecosystems that farmers must manage in order to produce fish crops.

The increase in human population coupled with large numbers of undernourished people, especially in developing countries, have made the need for food production a major worldwide issue of concern (Okechi, 2004). Studies have shown that there is a limit to world's natural stocks of fish and shellfish, though renewable, have finite production limits, which cannot be exceeded even under the best management regimes. Hence, the maximum sustainable fishing limit in natural waters has been exceeded (FAO, 2000). Therefore, fish production will depend on aquaculture to bridge the demand-supply gap of fish. According to (FAO, 2009) production in capture fisheries is stagnating and aquaculture output is expanding faster than any other animal-based food sector worldwide, particularly in developing countries. The rearing of the African catfish in Africa started in the early seventies in Central and Western Africa as it was realized that it was a very suitable species for aquaculture as it grows fast and feeds on a large variety of agriculture by products, is hardy and can tolerate

adverse water quality conditions among other qualities.

In fish farming, water plays a very important role and quality of the water supplied is important in determining the quality of the fish and the health of its consumer as well as the entire ecosystem.

Safe water quality is a major concern with reference to public health importance in view of the One Health Initiative which is a transdisciplinary approach that recognizes that the health of people is closely connected to the health of animals and our shared environment.

The physical characteristics of a fish pond directly impact pond water quality and indirectly the whole ecosystem and therefore, production management is potential for the farmers. The foregoing is one of the most overlooked aspects of good management. Shoko *et al.*, (2014) believed that production is reduced when the water contains contaminants that can impair development, growth, reproduction or even cause mortality to the cultural species. Some microorganisms reportedly isolated from pond water include *Pseudomonas* species, *Klebsiella* species, *Proteus* species, *Bacillus* species, *Micrococcus* species, *Aspergillus* species and *Penicillium* species (Daboor, 2008). To have successful aquaculture, there is a need for healthy fish and proper water quality management as a deficiency in any variable will reduce the growth and affect the health of the fish.

As noted by Bhatnagar and Devi (2013), water quality is made up of physical (temperature, density) chemical (pH, conductivity, nutrients) and biological (bacteria, plankton and parasites) which influence the use of water in fish culture purposes. Non-optimum water physicochemical parameters (dissolved oxygen, pH, salinity, ammonia, temperature, etc.) and poor management practices (overfeeding, inadequate nutrition, overcrowding, etc.) can cause stress to the cultured fish and thus make them more susceptible to disease outbreaks. This study aim at bacteriological assessment of fish and pond

water in fish farms that use chicken droppings as feeds in Keffi, Nigeria

## Materials and Methods

### Study Area

The study was carried out in Keffi, Nasarawa State. Keffi is located in the middle belt of Nigeria. It is geographically situated on latitude 8°50'N and longitude 7°52'E. Keffi town is on latitude 85° above the sea level and it is in the north west of Lafia, the state capital of Nasarawa State. It is 53km away from Abuja (capital of Nigeria) in the guinea savannah of Nigeria (Akwaet al., 2007).

### Sample Collection

Water samples were collected from fish ponds stocked with the African catfish (*Clariasgaripepinus*) sited at different locations in Keffi Local Government Area, Nasarawa State, Nigeria. A total of forty-eight (48) samples- pond water, thirty-six (36) and fish, twelve (12) were collected from four (4) different fish ponds. One time sampling in triplicate was carried out in each pond. The collection of water samples was from a depth of about 25 cm beneath the surface of the ponds (Odesiri-Eruteyan *et al.*, 2022). Before collection, sterile sampling bottles were thoroughly washed and rinsed with the same water to be collected from the ponds. The samples were collected using sterile screw-capped labelled bottles. The water samples were transported to the laboratory in an ice-packed container for bacteriological analyses between 2-3 h after collection.

### Preparation of Samples

The bacteriological analysis of the pond water and fish samples was carried out by serial dilution. 1ml of the diluents  $10^6$  was inoculated on nutrient agar plates for the isolation of bacteria and incubated at 37°C for 24 hours. The bacterial growth was counted in colony forming units. Sterile cotton swabs were used to collect the fish skin contents; these were placed in peptone water

and incubated overnight in aerobic conditions (Rivas *et al.*, 2015). The pre-enriched samples were then inoculated on Nutrient Agar where the bacterial growth was counted in colony forming units

### Isolation and Enumeration of Bacteria

#### Bacteriological analysis

The heterotrophic bacterial plate counts of the respective pond water and fish samples were evaluated using serial dilution and pour plate techniques as described by Cullimore (2000). Nutrient agar (NA) was used in the heterotrophic bacterial enumeration. Total coliform Count was analyzed using MacConkey agar and Faecal Coliform was analyzed using Eosin methylene blue agar using spread plate. The colonies were counted and recorded as colony forming units per milligram (cfu/ml) of effluent.

After incubation, resultant bacterial colonies from the plates were picked and purified by sub-culturing. Pure colonies obtained were maintained on Nutrient agar slants for further characterization and identification.

#### Identification of bacteria Isolated

The bacteria Isolated were identified using gram staining and biochemical tests which include catalase test, oxidase, indole test, citrate utilization test and hydrogen sulphide production test. Characterization and identification of the bacteria isolates was done by physical macroscopic examination of their cultural characteristics such as texture, color and other morphological characteristics. Biochemical analyses were carried out for further identification and characterization with reference to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

#### Phenotypic Assessment of Bacterial Isolates with Virulence Traits

Virulence traits such as production of hemolysin, survival in low pH, survival in blood serum and

binding with Congo Red dye was assessed using phenotypic methods.

### Congo Red dye binding

The Congo Red dye binding was performed using a Congo Red agar method as described by Freeman *et al.*, (1989). The isolates were cultivated into plates containing Congo red agar and incubated for 24h at 37°C under aerobic conditions. Biofilm producing isolates formed black colonies with a dry crystalline consistency, while the non-biofilm producer ones formed red colonies with a smooth and darkened appearance in the center.

### Survival in Low pH Assay

Cultures were grown to the appropriate phase of growth in tryptic phosphate broth (TPB) at 30°C and pH 7.0 with vigorous aeration. Viable cell counts were performed immediately and subsequently as the pH was being adjusted and at suitable time intervals (Mazzotta, 2001). Cultures were acid challenged by reduction of the medium pH to pH 3.0 with 10 M HCl. Serial dilution was performed in 0.1% peptone, and 10 µl of each dilution was spread onto BHI agar plates, which was incubated at 30°C for 24 to 48h. Volumes of culture between 10 µl and 1 ml were plated directly from the challenge medium. All survival experiments were performed at least three times.

### Survival in Blood Serum Assay

The serum resistance assay was performed by the method described by Kumar and Mathur (1997). The test organisms were grown in separate nutrient broth at 30°C overnight. The turbidity of the overnight broth culture was matched with McFarland standard (0.5 Brown's opacity tubes) and then adjusted to a count of  $10^4$ cfu/ml in 5ml fresh nutrient broth and incubated at 30°C for 2 hours. Cultures were centrifuged at 1500g for 5min and the deposit resuspended in 5ml sterile phosphate buffered saline (PBS). Equal volume (0.5ml each) of pooled normal human serum and bacteria suspension was mixed and incubated in a water bath at 30°C. Viable count was performed

on the pooled normal human sera and the bacteria suspension mixture at 0 and 3 hours using the surface spreading method. Where the viable count dropped to less than 1% of the initial value (comparing counts at 0 and 3 hours), the isolates were regarded as sensitive. However, where more than 90% of the organism survived after 3 hours, the isolate was said to be resistant. For counts between 1 and 90%, the isolate was termed intermediate.

### Hemolysis Assay

The bacteria isolate was subjected to a hemolytic test by streaking them onto trypticase soy agar plates supplemented with 7% whole blood (Brumfield *et al.*, 2018). The hemolytic zones was observed after incubation of plates at 30°C for 48 h.

## Results

### Total Bacteria Load Count from Different Fish Pond Water in Keffi

The total bacteria load count from the various fish pond water is as given in Table 1. From this analysis, the total viable counts of pond water from Pond A, B, C and D was  $29.03 \times 10^6 \pm 1.02$ ,  $23.12 \times 10^6 \pm 2.43$ ,  $18.66 \times 10^6 \pm 0.34$  and  $13.32 \times 10^6 \pm 0.73$  respectively. Also, the total coliform count for pond water from Pond A, B, C and D was  $21.11 \times 10^6 \pm 1.56$ ,  $13.28 \times 10^6 \pm 2.16$ ,  $9.44 \times 10^6 \pm 0.34$  and  $7.08 \times 10^6 \pm 0.44$ . The total fecal count of pond water from Pond A, B, C and D was  $16.43 \times 10^6 \pm 0.21$ ,  $8.78 \times 10^6 \pm 0.38$ ,  $4.12 \times 10^6 \pm 0.98$  and  $5.00 \times 10^6 \pm 0.86$  respectively.

### Total Bacteria Load Count from Fish Skin from Different Fish Ponds in Keffi

The total bacteria load count from the fish samples is as given in Table 4.2. From this analysis, the total viable counts of fish samples from Pond A, B, C and D were  $9.11 \times 10^6 \pm 0.22$ ,  $12.12 \times 10^6 \pm 0.63$ ,  $6.06 \times 10^6 \pm 0.10$  and  $6.21 \times 10^6 \pm 0.13$  respectively. Also, the total coliform count for pond water from Pond A, B, C and D was  $6.01 \times 10^6 \pm 0.026$ ,  $8.28 \times 10^6 \pm 2.66$ ,  $5.14 \times$

$10^6 \pm 0.03$  and  $4.28 \times 10^6 \pm 0.31$ . The total fecal count of pond water from Pond A, B, C and D

was  $5.43 \times 10^6 \pm 0.11$ ,  $5.18 \times 10^6 \pm 0.18$ ,  $3.67 \times 10^6 \pm 0.19$  and  $4.08 \times 10^6 \pm 0.23$  respectively.

**Table 1: Total Bacteria Load Count from Different Fish Pond Water in Keffi**

Water sources	Mean $\pm$ SD		
	Total viable count $\times 10^6$	Total coliform count $\times 10^6$	Total faecal count $\times 10^6$
Fish pond A	29.03 $\pm$ 1.02	21.11 $\pm$ 1.56	16.43 $\pm$ 0.21
Fish pond B	23.12 $\pm$ 2.43	13.28 $\pm$ 2.16	8.78 $\pm$ 0.38
Fish pond C	18.66 $\pm$ 0.34	9.44 $\pm$ 0.34	4.12 $\pm$ 0.98
Fish pond D	13.32 $\pm$ 0.73	7.08 $\pm$ 0.44	5.00 $\pm$ 0.86

**Table 2: Total Bacteria Load Count from Fish Skin from Different Fish Ponds in Keffi**

Fish sources	Mean $\pm$ SD		
	Total viable count $\times 10^6$	Total coliform count $\times 10^6$	Total faecal count $\times 10^6$
Fish pond A	9.11 $\pm$ 0.22	6.01 $\pm$ 0.026	5.43 $\pm$ 0.11
Fish pond B	12.12 $\pm$ 0.63	8.28 $\pm$ 2.66	5.18 $\pm$ 0.18
Fish pond C	6.06 $\pm$ 0.10	5.14 $\pm$ 0.03	3.67 $\pm$ 0.19
Fish pond D	6.21 $\pm$ 0.13	4.28 $\pm$ 0.31	4.08 $\pm$ 0.23

**Cultural, Morphology and Biochemical Characteristics of Bacterial Isolated From Fish Pond Water and Fish Skin**

Results for cultural, morphology and biochemical characteristics of bacterial isolates obtained from this study are as shown in Table 3. Gram negative cocci rod, catalase positive, coagulase negative, indole positive, oxidase positive, greenish colonies on EMB were suspected to be *E. coli*. Gram negative rod, catalase positive, coagulase negative, indole negative, oxidase positive smooth whitish colonies on NA with black deposit on SSA were suspected to be *Salmonella* species. Gram negative rod, catalase negative, coagulase negative, indole positive, oxidase positive colorless lactose-negative colonies, dark pinkish on MCA and Brown colored colonies without metallic sheen on EMB agar were suspected to be *Citrobacter* species. Gram negative rod, catalase positive, coagulase positive, indole negative,

oxidase positive pinkish colonies on both MAC and EMB agar were suspected to be *Klebsiella* species.

**Percentage Occurrence of Bacteria Isolated from Selected Fish Pond Water in Keffi**

The percentage occurrence of bacteria species isolated from selected fish pond water in Keffi is as given in Table 4. The bacteria isolated from pond A were *Klebsiella* spp (44.4%), *Citrobacter* spp (33.3%), *Salmonella* spp (11.1%) and *E. coli* (44.4%). The bacteria isolated from pond B were *Klebsiella* spp (22.2%), *Salmonella* spp (22.2%) and *E. coli* (33.3%). The bacteria isolated from pond A were *Citrobacter* spp (22.2%), *Salmonella* spp (33.3%) and *E. coli* (55.5%). The bacteria isolated from pond D were *Klebsiella* spp (55.5%), *Citrobacter* spp (33.3%), *Salmonella* spp (11.1%) and *E. coli* (22.2%) respectively.

**Table 3: Cultural, Morphology and Biochemical Characteristics of Bacterial Isolated from Fish Pond Water and Fish Skin**

Cultural Characteristics	Morphological Characteristics	Biochemical characteristics							Inference
		GS	CAT	COA	IN	VP	MR	OX	
Greenish colonies on EMB	Cocci rod	-	+	-	+	+	-	+	<i>E. coli</i>
Smooth Whitish on NA, black deposit on SSA	Rod	-	+	-	-	+	-	+	<i>Salmonella</i> spp
Colorless lactose-negative colonies, dark pinkish on MSA and Brown colonies without metallic sheen on EMB agar	Rod shape	-	-	-	+	-	-	+	<i>Citrobacter</i> spp
Pinkish on MAC pinkish on EMB agar	Rod	-	+	+	-	-	+	+	<i>Klebsiella</i> spp

KEY: P= pigment, MP= morphology, GS= gram staining, CAT= catalase, COA= coagulase, IN=indole, VP= Vogesproskauer, MR= methyl red, OX= oxidase, CT = citrase, + = positive, - = negative, MSA= mannitol salt agar, EMB= eosin methylene blue

**Table 4: Percentage Occurrence of Bacteria Isolated from Selected Fish Pond Water in Keffi**

Fish ponds	No. sample	No. (%) isolated			
		<i>Klebsiella</i> spp	<i>Citrobacter</i> spp	<i>Salmonella</i> spp	<i>E. coli</i>
A	9	4(44.4)	3(33.3)	1(11.1)	4 (44.4)
B	9	2(22.2)	0(0.0)	2(22.2)	3 (33.3)
C	9	0(0.0)	2(22.2)	3(33.3)	5 (55.5)
D	9	5(55.5)	3(33.3)	1(11.1)	2 (22.2)
Total	36	11 (30.5)	8 (22.2)	7(19.4)	14(38.8)

**Percentage Occurrence of Bacteria Isolated from Fish Skin of Selected Fish Pond Water in Keffi**

The percentage occurrence of bacteria species isolated from selected fish samples in Keffi is as shown in Table 5. The bacteria isolated from pond A were *Klebsiella* spp (66.6%), *Citrobacter* spp (33.3%), *Salmonella* spp (66.6%) and *E. coli*

(100.0%). The bacteria isolated from pond B were *Klebsiella* spp (33.3%) and *E. coli* (33.3%). The bacteria isolated from pond A were *Klebsiella* spp (100.0%), *Citrobacter* spp (66.6%) and *Salmonella* spp (33.3%). The bacteria isolated from pond D were *Citrobacter* spp (33.3%), *Salmonella* spp (33.3%) and *E. coli* (66.6%) respectively.

**Haemolysin Production by Bacteria Isolated from Selected Fish Pond Water and Fish Skin**

The result of the hemolysin production assay is as shown in Table 6. For the pond water samples, 42.8% of *Salmonella* spp, 50.0% of *Citrobacter* spp, 45.4% of *Klebsiella* spp and 21.4% of *E. coli* under investigation exhibited halo zones. Also, for the fish samples, 50.0% of *Salmonella* spp, 33.3% of *Citrobacter* spp, 33.3% of *Klebsiella* spp and 66.6% of *E. coli* under investigation exhibited halo zones.

**Congo Red Dye Binding by Bacteria Isolated from Selected Fish Pond Water and Fish Skin**

The result of the Congo red dye binding assay is as shown in Table 7. For the pond water samples, 71.4% of *Salmonella* spp, 37.5% of *Citrobacter* spp, 54.5% of *Klebsiella* spp and 57.1% of *E. coli* binded with Congo Red dye. Also, for the fish samples, 75.0% of *Salmonella* spp, 66.6% of *Citrobacter* spp, 50.0% of *Klebsiella* spp and 66.6% of *E. coli* binded with Congo Red dye.

**Table 5: Percentage Occurrences of Bacteria Isolated from Fish Skin of Selected Fish Pond in Keffi**

Fish ponds	No. sample	No. (%) isolated			
		<i>Klebsiella</i> spp	<i>Citrobacter</i> spp	<i>Salmonella</i> spp	<i>E. coli</i>
A	3	2(66.6)	0(0.0)	2(66.6)	3(100)
B	3	1(33.3)	0(0.0)	0(0.0)	1(33.3)
C	3	3(100)	2(66.6)	1(33.3)	0(0.0)
D	3	0(0.0)	1(33.3)	1(33.3)	2(66.6)
Total	12	6(50.0)	3(25.0)	4(33.3)	6(50.0)

**Table 6: Haemolysin Production by Bacteria Isolated from Selected Fish Pond Water and Skin**

Isolates	Fish Pond water	Halo zone (%)	Fish skin	Halo zone (%)
<i>Salmonella</i> spp	7	3(42.8)	4	2(50.0)
<i>Citrobacter</i> spp	8	4 (50.0)	3	1(33.3)
<i>Klebsiella</i> spp	11	5 (45.4)	6	2 (33.3)
<i>E. coli</i>	14	3 (21.4)	6	4 (66.6)

**Table 7: Congo Red Dye Binding by Bacteria Isolated from Selected Fish Pond Water and Fish Skin in Keffi**

Isolates	Fish Pond water	No. (%) bind	Fish skin	No. (%) bind
<i>Salmonella</i> spp	7	5(71.4)	4	3(75.0)
<i>Citrobacterspp</i>	8	3 (37.5)	3	2(66.6)
<i>Klebsiellaspp</i>	11	6 (54.5)	6	3 (50.0)
<i>E. coli</i>	14	8 (57.1)	6	4 (66.6)

**Survival in Blood Serum by Bacteria Isolated from Selected Fish Pond Water and Fish Skin**

The result of the survival in blood serum assay is as shown in Table 8. For the pond water samples, 85.7% of *Salmonella* spp, 50.0% of *Citrobacter* spp, 63.6% of *Klebsiellaspp* and 42.8% of *E. coli* survived in blood serum. Also, for the fish samples, 50.0% of *Salmonella* spp, 66.6% of *Klebsiella* spp and 33.3% of *E. coli* survived in blood serum.

**Survival in Low pH by Bacteria Isolated Fish Pond Water and Fish Skin**

The result of the survival in low pH assay is as shown in Table 9. For the pond water samples, 57.1% of *Salmonella* spp, 25.0% of *Citrobacter* spp, 45.4% of *Klebsiella* spp and 28.5% of *E. coli* survived in blood serum. Also, for the fish samples, 25.0% of *Salmonella* spp, 33.3% of *Citrobacter* spp, 50.0% of *Klebsiella* spp and 50.0% of *E. coli* survived in blood serum

**Table 8: Survival in blood serum by Bacteria Isolated from Selected Fish Pond Water and Fish skin from Selected Fish Ponds in Keffi**

Isolates	Fish Pond water	No.(%) Survival	Fish skin	No. (%) Survival
<i>Salmonella</i> spp	7	6 (85.7)	4	2 (50.0)
<i>Citrobacterspp</i>	8	4 (50.0)	3	0 (0.0)
<i>Klebsiellaspp</i>	11	7 (63.6)	6	4 (66.6)
<i>E. coli</i>	14	6 (42.8)	6	2 (33.3)

**Table 9: Survival in Low pH by Bacteria Isolated from Selected Fish Pond Water and Fish skin from Selected Fish Ponds in Keffi**

Isolates	Fish Pond water	No. (%) Survival	Fish skin	No. (%) Survival
<i>Salmonella</i> spp	7	4 (57.1)	4	1 (25.0)
<i>Citrobacter</i> spp	8	2 (25.0)	3	1 (33.3)
<i>Klebsiella</i> spp	11	5 (45.4)	6	3 (50.0)
<i>E. coli</i>	14	4 (28.5)	6	3 (50.0)

**Discussion**

Fish is man’s most important single source of high-quality protein, compared to other animal sources. It is relatively affordable and greatly suitable without religious prejudice, giving it an added benefit over other sources of proteins (Philips *et al.*, 2015 & FAO, 2000). The use of organic manure results in discharge of large volumes of microbes which are discharged into the surrounding drains leading to pollution of the environment and nearby household drinking water sources. This study showed that pathogens present in aqua facilities can thrive and persist in fish and pond water. The level of total viable bacteria

count from the pond water samples ranged from  $29.03 \times 10^6 \pm 1.02$  cfu/ml in Pond A to  $13.32 \times 10^6 \pm 2.43$  in Pond D. The highest total coliform count and faecal count from the pond water sample was  $21.11 \times 10^6 \pm 1.56$  cfu/ml and  $16.43 \times 10^6 \pm 0.21$  cfu/ml in Pond A concurrently.

Additionally, the level of total viable bacteria count from the fish samples ranged from  $12.12 \times 10^6 \pm 0.63$  cfu/ml in Pond B to  $6.06 \times 10^6 \pm 0.10$  cfu/ml in Pond C. The highest total coliform count and faecal count from the fish sample was  $8.28 \times 10^6 \pm 2.66$  cfu/ml and  $5.43 \times 10^6 \pm 0.11$  cfu/ml in Pond B and Pond A respectively. The total viable bacteria count, coliform and faecal



count observed from fish pond water and fish skin in this study was high but similar to the study reported by Orji *et al.*, (2022) where the highest total viable bacteria count, coliform and faecal count recorded was  $2.8 \times 10^5 \pm 0.01$ ,  $1.2 \times 10^3 \pm 0.10$  and  $0.5 \times 10^2 \pm 0.04$ . In this study, four different bacteria species of public health importance namely *Escherichia coli*, *Salmonella enteric* serovar *Typhimurium*, *Citrobacter freundii* and *Klebsiella pneumonia* were identified. These pathogenic bacteria isolated have been implicated in infections which may range from invasive infection to toxin infection in humans. *Salmonella enterica* serovar *Typhimurium* is one of the most common causes of food-borne illness and is a major cause of diarrheal diseases (Kabiret *al.*, 2012 and Kariuki *et al.*, 2006). *Klebsiella pneumoniae* is an important opportunistic and gram-negative bacterium that causes infections in the respiratory tract, circulatory system, urinary tract, and wounds in people with underlying disease (Chang *et al.*, 2021). *E. coli* causes human illnesses characterized by hemorrhagic colitis, vomiting, nausea, and other agent-related symptoms (Mumbo *et al.*, 2023). *Citrobacter freundii* and *C. braakii* is considered an opportunistic pathogen that is commonly associated with food borne diseases, bloodstream infections, intra-abdominal sepsis, brain abscesses, pneumonia, and other neonatal infections such as meningitis, neonatal sepsis, joint infections, or common bacteremia (Ashishet *al.*, 2012, Hawaldar and Sadhna, 2019 and Räsänen *et al.*, 2021).

Also, *C. freundii*, *Klebsiella* spp, *Salmonella* spp and *E. coli* are zoonotic bacteria that are present in fish as hosts and cause infection in fish (Lüet *al.*, 2011 & Altun *et al.*, 2013). *Citrobacter braakii* and *C. freundii* causes diseases in fish which associated with hemorrhagic septicemia, severe enteritis, severe kidney disease and gill lesions of catfish (De Pádua *et al.*, 2014 & Bandeira *et al.*, 2018). Ghosh & Bandyopadhyay (2019) reported that bacteriological studies on catfish revealed that *Klebsiella* is the most common pathogen causing prevalence of ulcers, fin erosion, and other lesions. Also, Udeze *et al.*, (2012) observed that upon inoculation of

*Clarias gariepinus* (catfish) with *Klebsiella pneumoniae*, virulent in the fishes leading to shedding of skin patches and fading of colour on skin from black to faint black were observed.

*Salmonella enterica* serovar *Typhimurium* is not a normal bacteria component of fish microbial flora. However, some studies have demonstrated the high incidence of *Salmonella* in fish intestines, skin and gills (Nwiyi & Onyeabor, 2012). Upon contamination, fish may become *Salmonella* hosts without presenting clinical manifestations (Bibi *et al.*, 2015). The occurrence of this pathogen is commonly related to its breeding environment. Another factor contributing to *Salmonella* contamination in fish is the use of poultry litter as feed in culture tanks (Ampofo & Clerks, 2010; Esposto *et al.*, 2007). *Escherichia coli* do not naturally occur in fish microbiota. It can however be transferred to fish via contaminated aquatic environments (Guzmán *et al.*, 2004). *E. coli* is a harmful bacterium found naturally in the stomach and intestine of fish that causes sickness (Austin *et al.*, 2007). *E. coli* isolates were found among the fish infected with dropsy, ulcer, red spot, and hemorrhagic septicemia infections (Hassan and Ali, 2024).

Detection of virulent traits using phenotypic methods were used to determine haemolysin production, serum resistance, low pH resistance and Congo red binding. Phenotypic detection of haemolysin production, survival in blood serum, survival in low pH and Congo Red binding was exhibited by 41%, 53%, 40% and 58% of the bacteria isolates respectively. The production of haemolysin by these bacteria isolates suggest that the isolates may encode a haemolysin pore forming toxin, that is responsible for virulence that leads to systemic infections development (Clarke *et al.*, 2006). The high Congo Red dye binding recorded in this study suggested that these bacteria isolated from different water sources have the ability of virulence factors, such as adherence (Rajkumar *et al.*, 2016). Bacteria with adherence genes had strong biofilm-formation ability. This virulence factor is an essential unit for protecting the bacteria against the host immune system thereby causing human infection

(Chikezie, 2012). Pathogens that evade innate immunity such as blood serum as demonstrated in this study pose a threat to human health. Also, their ability to evade innate immunity and survive in growth-restricted pH increases the likelihood of infection significantly (Acosta & Alonzo, 2023).

## Conclusion

This study showed that pathogenic bacteria were from fish and pond water used chicken droppings as feed in their fish farms in Keffi. This may lead to transmission of foodborne diseases when improperly cooked fishes are consumed thereby jeopardizing human's health and also fouling the environment upon discharge of the pond water into the environment. Although population increase and related demand for food (especially animal protein) and a growing recognition of the resource value of poultry waste and nutrients it contains are some of the driving forces increasing the use of animal waste in fish farming, the adverse impact on human health and the environment should be put into consideration as the poor and unhygienic individuals bear the heaviest burden of disease transmission through faecal-oral route. Adequate education on protocol for disinfection of fish pond effluent and proper disposal of diseased populations of aquatic animals should be included in the SOP of every aquaculture facility. By managing the disposal of fishpond water sustainably, production of food and energy will be better managed thus contributing decent work and economic growth.

## Conflict of Interest statement

The authors declare no conflicts of interest

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