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Microbiological Evaluation of Fresh Catfish (*Clarias gariepinus*) Obtained from Selected Markets and Ponds in Minna Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aimed at evaluating the microbiological quality of fresh catfish (*Clarias gariepinus*) sold in three different markets and ponds in Minna metropolis. The total plate counts from Bosso pond are 2.7×10^2 cfu/mL and 3.8×10^2 cfu/mL (satisfactory) for skin and gills respectively. Kure pond includes total plate counts of 4.7×10^3 cfu/mL and 3.3×10^2 cfu/mL (satisfactory) for skin and

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gills respectively while Mobil pond had total plate counts of 2.5×10^3 cfu/mL and 2.5×10^2 cfu/mL on skin and gills respectively. The total coliforms counts from Bosso pond are 3.8×10^2 cfu/mL and 3.0×10^1 cfu/mL (satisfactory) for skin and gills respectively. Kure pond includes total coliforms counts of 2.4×10^2 cfu/mL and 2.6×10^2 cfu/mL (satisfactory) for skin and gills respectively while Mobil pond had total coliforms counts of 4.6×10^3 cfu/mL and 3.2×10^4 cfu/mL on skin and gills respectively. The coliforms count of fresh catfish (*Clarias gariepinus*) obtained from three markets locations are total coliforms counts from Bosso market are 5.6×10^4 cfu/mL and 4.0×10^5 cfu/mL (Unsatisfactory) for skin and gills respectively. Kure market includes total coliforms counts of 1.6×10^3 cfu/mL and 7.2×10^3 cfu/mL (satisfactory) for skin and gills respectively while Mobil market had total coliforms counts of 6.0×10^4 cfu/mL and 4.8×10^4 cfu/mL (Marginal) on skin and gills respectively.

The fungal count of fresh catfish (*Clarias gariepinus*) obtained from three markets locations are Bosso market 1.3×10^2 cfu/mL and 1.5×10^1 cfu/mL (satisfactory) for skin and gills respectively. Kure market 2.0×10^3 cfu/mL and 1.0×10^2 cfu/mL (satisfactory) for skin and gills respectively while Mobil market had total fungal counts of 1.7×10^4 cfu/mL and 1.7×10^2 cfu/mL on skin and gills respectively.

The following bacteria were isolated *Escherichia coli, Bacillus substilis, Staphylococcus aureus, Pseudomonas species, Streptococcus species, Shigella species and Enterobacter species*. The fungi isolated were *Rhizopus spp* and *Aspergillus niger.*

Keywords: Microbiological; catfish (Clarias gariepinus); markets; ponds.

1. INTRODUCTION

Fish is one of the major animal protein foods in the tropics [1]. It is particularly valuable for providing proteins of high quality comparable with those of meat, milk or eggs. The African catfish *(Clarias gariepinus*) is easily cultured in Nigeria and is of great economic interest. Adewumi et al*.* [2]. *Clarias gariepinus* is generally considered to be one of the most important tropical catfish species for aquaculture. The last two decades have seen appreciable increase in global fish trade and the need to enforce safety standards and regulations on imported consignment especially from developing nations fraught with unacceptable levels of microbiological contamination [3].

Many species of fish (approximately 6850) live in fresh water lakes and rivers. Fish is mainly thought of as a source of proteins [4]. Fishing in Minna metropolis is mainly done by the Gwaris and Gbagyl tribes and the women are responsible for the sale especially in Shiroro and Gurara locals in Niger state of Nigeria. The microorganisms associated with fresh catfish harvested from rivers and ponds in Minna is essential in order to establish an overview of the bacteriological pathogens associated with fresh fish in Niger state Nigeria and to compare with other parts of the world [4].

Spoilage patterns of fish have been well documented [5] by its sizes. Fresh fish spoilage and high perish ability are primarily due to large amount of non-protein nitrogen (like free amino acids), volatile nitrogen bases (ammonia, creatine, taurine, uric acid, carnosine and histamine) which support post mortem bacteria growth.

Fish have been known to harbor micro flora. Gill [6]; Jacosen and Lisbon [7].These are microorganisms are found mostly in their gills and gut. Microbes may become intimately associated with the external surfaces of the fish or may be incorporated into the resident micro flora [8]. The catfish species in Nigeria are *Clarias gariepinus, Heterobanchus bidorsalis*, *Clarias heterobranchus hybrid* (*Heteroclarias*), and *Clarias nigrodigitatus*. Ichiba et al., Ohuoba et al. [9].

Heterobranchus species is the most commonly cultured fish in south eastern part of Nigeria; African catfish is popular in the market and has great potential to boost the rapidly growing Nigerian aquaculture. Generally, the fact that fish farming affords best utilization of land and provides income to an investor; it is one of the cheapest sources of animal protein. FAO [10].

Fish spoilage is one of the greatest problems affecting the fishing industry worldwide and attempts are being made to reduce the spoilage to the barest minimum. About 20 to 50% of

domestic fish productions are wasted through spoilage in tropical developing countries [11].
The broad objective of this work is to evaluate the microorganisms associated with cat fish *(Clarias gariepinus*) fish species obtained from selected markets and ponds in Minna metropolis. To estimate the microbial load of fresh catfish obtained from the ponds and markets in Minna metropolis, to isolate and identify micro-organisms associated with fresh catfish obtained from the ponds and Markets location in Minna metropolis.

2. MATERIALS AND METHODS

2.1 Sources of Materials and Preliminary Handling

Samples of fresh catfish (*Clarias gariepinus)* about 1.5 m long and weighing 10 g were collected. Fresh catfish were randomly selected and purchased from Kure market, Mobil fish market and Bosso market on various occasions, 9.00 a.m in a specific time of the day. These samples were conveyed in different polythene bags surrounded by ice block beside the polythene bags to the laboratory were the practical was carried out and the sample was kept in the freezer for about 2 h before analysis were carried out on each species.

2.2 Method of Analysis

The bacteriological media and reagent used were prepared and sterilized by autoclaving at temperature of about 121 ºC for 15 min. Sterile water (100 mL) was poured into each polythene bags containing one of each species and was used to thoroughly wash the skin of the fish. And 1.0 mL of wash water was diluted to 10 fold in phosphate buffered saline .Various culture media are namely: Nutrient agar, Mac Conkey agar, and Potato Dextrose Agar (P.D.A) were used. Others were test tube, string (injection needle), cotton wool, standing cork, and Petri-dishes. The gills were aseptically removed and weighed (about 1.3 g) in various sample and recorded and was similarly treated like the skin. Anthonio and Akinwumi [12].

2.3 Procedure for Preparing the Media

2.3.1 Nutrient agar

Nutrient agar was used for cultivation of bacteria. It contains beef extract 3.0 g, agar-agar 15 g, gelysate peptone 5.0 g, final pH of 6.8 obtained. Thereafter, 28.0 g of nutrient agar was dissolved in one litre (1000 mL) of distilled water, and then sterilized at 121ºC for 15 min.

2.3.2 Potato dextrose agar (PDA)

Twenty four grams of P.D.A powder were dissolved in 500 mL of distilled water and allowed to dissolve gently it was later autoclaved at 121ºC for 15 min.

2.3.3 Mac Conkey agar

Twenty four grams of Mac Conkey agar were dissolved in 500 mL of distilled water and autoclaved at 121ºC for 15 min.

2.4 Serial Dilution

Nine millilitres of distilled water was dispensed into test-tube and sterilized by autoclaving at 121 ^{0}C for 15 min. It was allowed to cool, five in number; for each sample carried out (i.e. skin and gill for every sample). 1.0 mL of sample washed from the skin was dispensed into the test-tube serially and mixed thoroughly and label 10^{-1} and introduced into the second test-tube. It was repeated up to 10^{-5} for each sample. Anthoni et al. [13]; Dalgaard [14].

1.0 mL was also pipette from each test-tube and placed in petri-dishes and 15.0 mL of nutrient agar was added to it and rocked on the table to mix, and allow to gel. Then the petri-dishes were taken to incubator and incubated at 30^{0}C for 48 to 72 h. The plates were placed in colony counter and recorded as colony forming units per gram. The micro organisms obtained were sub-cultured and isolated in universal bottles for identification.

2.5 Fungi

Serial dilution for fungi culture procedure is the same with the above but potatoes dextrose agar was used instead of nutrient or Mac Conkey agar.

2.6 Bacterial Loads

2.6.1 Total and viable plate counts

The plate count method was employed for the enumeration of bacterial cells in the sample. Plates with count ranging from 30 to 300 colonies were considered for population estimation. Therefore, a dilution which yielded fewer than 300 colonies per plate were selected since

colonial concentrations exceeding this count was usually been depressed to an unknown degree by overcrowding and micro antagonism. Harrigan and McCance [15].

2.6.2 Enumeration of micro organisms

Samples were taken for total number of living and dead cells; The number of living organism per mL in this technique was used to assess the quality of the sample. Clucas [16].

2.7 Bacteria Gram Stain Reaction

2.7.1 Gram stain

The Gram stain was done as the first step in diagnosis of a bacterial infection.

2.7.2 Steps carried out to perform gram staining of samples

Two drops of the tissue sample were added onto a glass slide

↓

The smear was heat fixed, by quickly passing it two to three times through a flame

↓

The smear was flooded with crystal violet / gently rinsed off the crystal violet with tap water

↓

The smear with iodine was flooded / and the iodine was gently rinsed off with tap water

↓

It was decolorized by adding alcohol /acetone to the smear while holding the slide at an angle to allow the decolourizer to drain

↓

The excess decolourizer was rinsed off with tap water

↓

The smear was flooded with safranin counter stain / gently rinsed off excess safranin with tap water

↓

The slide was drained and allowed to air dry

↓

The slide was examined under the light microscope

Fig. 1. Flow diagram of gram staining bacteria

2.7.3 Indole test

Materials included: Test tube containing 1 % peptone water, bacterial culture, kovac's reagent.

The peptone water was inoculated with the bacterial culture and incubated for 48 h at 37^0C. 0.5 mL of kovac's reagents was added and shook gently.

Presence of red color indicated indole (positive).Absence of red color indicated not indole (negative).

2.7.4 Citrate Utilization test

This was done to identify some members of the family *Enterobacteriaceae.* Citrate was the only carbon source available to the bacteria. The media turned a bright blue as a result of an increase in the pH of the media. The transfer loop was used. The citrate agar was inoculated and incubated at 30 ^{0}C for 72 h for organism to grow.

2.7.5 MR-VP (Methyl red-Vogues Proskauer)

The MR portion (methyl red) was used to determine if glucose can be converted to acidic products like lactate, acetate, and formate. The VP portion was used to determine if glucose can be converted to acetoin.

These tests were performed by inoculating a single tube of MR-VP media with a transfer loop and then allowed to grow for 3 days. After the culture was grown, about half of the culture was transferred to a clean tube. One tube of culture was used to conduct the MR test, the second tube serves as the VP test.

MR (Methyl red) test: Methyl red was added to the MR tube. A red color indicated a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate. A yellow colour indicated a negative result; glucose was converted into neutral end products.

First alpha-napthol (also called Barritt's reagent A) and then potassium hydroxide (also called Barritt's reagent B) were added to the VP tube. The culture was allowed to sit for about 15 min for colour development to occur. If acetone was produced then the culture turns a red colour (positive result); if acetoin was not produced then the culture appeared yellowish to copper in colour (a negative result). Alverson [17].

2.7.6 Catalase

This test was used to detect the enzyme catalase. This enzyme was responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. 0.5 mL of the samples was smeared unto the lid of a petri plate/culture dish. Two drops of hydrogen peroxide were added to the smear. Bubbles became visible and the test was positive to catalase. A lack of bubbles indicated the absence of catalase.

Equation

$$H_2O_2 \ _{+\ \text{Organism}} \quad \underline{\text{catalase}} \rightarrow \quad H_2O + O_2$$

2.7.7 Coagulase test

This was performed to differentiate positive from negative bacterial in the presence of coagulation.

Procedure carried out:

- A drop of physiological saline was placed on each clean slide.
- Emulsified a colony of the test organism with wire loop on each of the drops to make thick suspensions.
- Two drops of plasma was added to the suspensions and mixed gently. Clumping of organism within 10 s was observed.
- Presence of agglutination gave positive.
- Absence of agglutination gave negative.

3. RESULTS AND DISCUSSION

3.1 Microbiological Load of Catfish sample from skin and gills

Table 1 shows the result for the total plate counts and coliforms counts on the skin and gills from various ponds and standards control limits. Table 2 shows the coliforms and fungal counts of fish samples from three different market locations in Minna metropolis. Table 3 shows the microscopic appearance, colonial morphology and biochemical characteristics of the bacterial isolated from fresh catfish in three markets/ponds locations in Minna.

The total coliforms unit count (cfu/mL) of fish sample in Bosso market is 5.6×10^4 cfu/mL and 4.0×10^5 cfu/mL gotten from skin and gills. The total coliforms counts on skin and gills of fish sample in Mobil market where 6.0×10^6 cfu/mL and 4.8×10^4 cfu/mL. The total coliforms unit counts in Kure market were 1.6×10^3cfu/mL and 7.2×10^3 cfu/mL gotten from skin and gills. The total number of fungi colony count found in catfish sample from both skin and gills found in

Table 1. Microbial loads of fish samples from three various ponds and standards control limits

Location of ponds	Parts isolated from	Total plate count (cfu/g)	Coliforms counts cfu/mL	Standards Organization of Nigeria(SON) 4×10^2 cfu/mL (AL)
Bosso	Skin	2.7×10^2	3.8×10^2	Satisfactory
	Gills	3.8×10^2	3.0×10^1	Satisfactory
Kure	Skin	4.7×10^3	2.4×10^2	Satisfactory
	Gills	3.3×10^3	2.6×10^2	Satisfactory
Mobil	Skin	2.5×10^3	4.6×10^3	Satisfactory
	Gills	2.5×10^2	3.2×10^4	Marginal

Data are means of triplicate determinations cfu/ml = colony forming unit per mill; AL = acceptable level, Satisfactory < 10^3; Marginal < 10^4 ; Unsatisfactory >10^5. SON=Standards Organization of Nigeria

Table 2. Coliforms and fungal counts of fresh catfish (*Clarias gariepinus*) obtained from three different markets locations

S/N	Location	Parts isolated from	Coliforms counts	Fungal counts	Standards Organization of Nigeria (SON) Acceptable Limits
			cfu/mL	cfu/mL	
1	Bosso	Skin	5.6×10^4	1.3×10^2	Marginal
		Gills	4.0×10^5	1.5×10^1	Unsatisfactory
2	Kure	Skin	1.6×10^3	2.0×10^3	Satisfactory
		Gills	7.2×10^3	1.0×10^2	Satisfactory
3	Mobil	Skin	6.0×10^4	1.7×10^4	Marginal
		Gills	4.8×10^4	1.7×10^2	Marginal

Data are means of triplicate determinations cfu/ml = colony forming unit per mill; AL = acceptable level, Satisfactory < 10^3; Marginal < 10^4 ; Unsatisfactory >10^5. SON=Standards Organization of Nigeria

Tables 3 and 4. Characterization and identification of bacteria isolate from three markets and ponds location

Table 3.

Location	Parts isolated from	Microscopic appearance			Colonial morphology				Biochemical tests		
		MCA	Shape	Elev/form	Gram reaction (mm)	Motility	MR-VP	Indole	Citrate	Catalase	Coagulase
Bosmkt	Skin	Pink	Rod	Convex irregular	-	-	+	-	+	-	+
	Gills	White	Rod	Convex irregular	+	+	+	-	+	+	+
Kurmkt	Skin	Pink	Cocci	Convex spindle	+	+	-	+	+	-	+
	Gills	Pink	Rod	Convex spindle	+	+	+	-	+	+	+
Mobmkt	Skin	Pink	Cocci	Convex Circular	-	-	-	+	-	-	-
	Gills	White	Rod	Spherical	+	+	+	-	+	+	+

Table 4.

Location	Parts isolated from	Micro appearance			Colonial morphology				Biochemical tests		
		MCA	Shape	Elev/form	Gram reaction (mm)	Motility	MR-VP	Indole	Citrate	Catalase	Coagulase
Bospond	Skin	Green	Rod	Spherical	-	+	+	-	+	-	-
	Gills	Pink	Cocci	Convex spindle	+	-	-	+	-	-	+
Kurpond	Skin	Green	Cocci	Convex spindle	+	-	-	+	-	+	+
	Gills	Pink	Rod	Convex spindle	+	+	-	+	-	-	+
Mobpond	Skin	Pink	Rod	Convex spindle	+	-	-	+	-	-	+
	Gills	White	Rod	Spherical	+	-	-	-	+	-	+

KEY: MR-VP=Methyl Red –Vogues Proskauer test, MCA= Mac Conkey Agar

Kure market were 2.0× 10^3cfu/mL and 1.0× 10^2cfu/mL while fungi colony found in Mobil market were 1.7× 10^4 cfu/mL and 1.7× 10^2 cfu/mL from both skin and gills. And fungi colony found in Bosso markets were 1.3× 10^2 cfu/mL and 1.5× 10^1 cfu/mL from both skin and gills respectively.

In summary therefore, Table 3 shows that the bacteria identified from isolate were *Escherichia coli, Staphylococcus specie, Streptococcus specie, Bacillus specie, Shigella specie, Enterobacter specie and Pseudomonas specie.*

4. DISCUSSION

4.1 Catfish Contamination at Various Markets

The result shown in Table 3 reveals that catfish species purchased from the various markets in Minna metropolis were highly contaminated with various bacterial species such as *Escherichia coli, Staphylococcus spp, Streptococcus spp, Bacillus, Shigella, Enterobacter spp and Pseudomonas* and fungi species were *Aspergillus spp* and *Rhizopus species* . This could have resulted due to the dirty environment of the markets or from the fish seller/ handlers themselves through their containers, sneezing and picking of noses. This is in consistence with the observation of Daniel et al*.* [4] who reported higher concentration of bacterial at various markets at Makurdi as compared to fresh fish samples caught at River Benue. However the total coliform count was highest on the skin than the gills from catfish obtained from Bosso market compared to that of Mobil and Kure market. This could be due to the concentration of broken soak ways and poor sewage disposal systems available there. Ariahu et al. [18].

4.2 Mould and Fungi Contamination at Various Markets

Aspergillus niger and *Rhizopus spp* probably contaminated the catfish sample through the air and soil, micro-organism are ubiquitous, [19-21]. These were not isolated from the central expert i.e. the catfish obtained from ponds. Adequate and proper cleaning detergents used by the fish reared may have ensured that there was no fungal nor mould contamination. *Aspergillus spp and Rhizopus spp* contaminated catfish samples from Bosso, Mobil and Kure markets. This could be due to inadequate personal hygiene.

5. CONCLUSION

The microbial load of the catfish (Clarias *gariepinus) are* varies according to difference in location and distance from purchasing of source. This has shown clearly from the data generated, the following species of organisms are the major containers; such species are *Escherichia coli, Staphylococcus spp, Streptococcus spp, Bacillus, Shigella, Enterobacter spp.* This contamination of fish occur probably due to conveyed of fish to the various markets or the material in which the fish is being kept on, it may also be as a result of been exposure of fish to flies and insects. This contamination may also result from dusty atmosphere and dirty environment, by heaps of refuse dump all over the place by the market seller.

6. RECOMMENDATION

Proper sanitation should be implemented in the fresh fish market and pond in order to maintain the hygienic environment. Awareness campaign needs to be conducted at fish selling location on mode of fish handing to reduce contamination. Further studies needed to be carried out in order to ascertain the point of entry of containers with a view of curtailing it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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