MICROBIOLOGICAL EVALUATION OF FRESH CATFISH (*Clarias gariepinus*) OBTAINED FROM SELECTED MARKETS AND PONDS IN MINNA METROPOLIS

ABSTRACT

Microbiological evaluation of fresh catfish (*Clarias gariepinus*) sold in three different markets and ponds in Minna Metropolis were carried out. The following bacteria were discovered *Escherichia coli, Bacillus spp, Staphylococcus spp, Pseudomonas species, Streptococcus species, Shigella species* and *Enterobacter species*. Bosso market skin *Escherichia coli, Staphylococ ,Aspergillus niger Streptococcus spp, Rhizopus specie* Kure market *Pseudomonas aeniginosa Escherichia coli Aspergillus Staphylococcus spp Aspergillus Bacillus subtilis,* Mobil market, *Escherichia coli Rhizopus spp, Staphylococcus aureus, Aspergillus flavus,* Bosso ponds *Enterobacter spp, Pseudomonas spp* , Mobil pond *Streptococcus spp* Kure pond *Escherichia coli* . Bosso pond (Skin) 3.8×10^2 Gills 3.0×10 (Satisfactory) Kure pond (Skin) 2.4×10^2 Satisfactory Gills 2.6×10^2(Satisfactory) Mobil pond The bacteria load count is ranged from 4.0×10^5 cfu/mL and 1.64×10^3 cfu/mL. The fungi isolated were *Rhizopus spp* and *Aspergillus niger*. The fungi count from the catfish sample is ranged from 0.5×10^1 cfu/mL and 2.0×10^3 cfu/mL. This shows that bacteria and fungi contaminations occurred in catfish obtained from three different markets location in Minna, Niger state.

Keywords: Microbiological, catfish (*Clarias gariepinus*), markets, ponds.

1.0 INTRODUCTION

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 (ICMSF, 2005).

Contamination concern has been on high loads of unsuspected spoilage by microorganisms like *Salmonella sp, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* (Gram *et al.,* 2000). Many spp of fish (approximately 6850) live in fresh water lakes and rivers. Fish is mainly thought of as a source of proteins.(Daniel *et al.,* 2006).Fishing in Minna metropolis is mainy done by the Gwaris and Gbagyl tribes and the women are responsible for the sale expecially in Shiroro and Gurara locals in Niger state of Nigeria.The microorganisms associated with fresh catfish in harveste from rivers and ponds in Minna is essential in order to establish an overview of the bacteriological pathogens associated with fresh fish in Niger and t cmpare with other parts of the world. (Daniel *et al.,* 2006)

Spoilage patterns of fish have been well documented (Botta, 2007) its size. 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 (Jay *et al.,* 2005).

Acuff and Finne reported that heating, freezing and exposure to high concentration of salt lead to chemical and physical changes, and therefore digestibility increased due to protein denaturation, but content of thermo labile compound and polyunsaturated fatty acid is often reduced.

Fish have been known to harbor micro flora. These are microorganisms and 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 (Austin and Austin, 1987). The catfish species in Nigeria are *Clarias gariepinus, Heterobanchus bidorsalis*, Clarias *heterobranchus hybrid* (*Heteroclarias*), and *Clarias nigrodigitatus*.

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 (Olaleye and Adewumi 2010). 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.

Fish spoilage is one of the greatest problems affecting the fishing industry world wide 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 Eyo (2001)

The broad objective of this work is to evaluate the microorganisms associate with cat fish *(Clarias gariepinus*) sample obtained from selected ponds in Minna metropolis. To estimate the microbial load of fresh catfish obtained from the pond and market in Minna Metropolis, to isolate and identify micro-organisms associated with fresh catfish obtained from the ponds and Markets location in Minna metropolis.

2.0 MATERIALS AND METHODS

2.1 Sources of materials and preliminary handling

Samples of fresh catfish *Clarias gariepinus*. Fresh fish were randomly selected and purchased from Kure market, Mobil fish market and Bosso market on various occasions, 9.am in a specific time of the day. These samples were conveyed in different polythene bags surround 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 hours 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°C for 15min. 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 gill were aseptically removed and weighed in various sample and recorded and was similarly treated like the skin.

2.2.1 Procedure for preparing the media

2.2.2 Nutrient agar

Nutrient agar was used for cultivation of bacteria, it is called general purpose agar medium. it contain beef extract 3.0 g, agar-agar 15 g, gelysate peptone 5.0 g, final pH of 6.8. Therefore 28 g of nutrient agar was dissolved in one liter (1000ml) of distill water, and then sterilized at 121 °C for 15 min.

2.2.3 Potato dextrose agar (PDA)

Twenty four grams of P.D.A powder were dissolved in 500 mL of distilled water allow it to dissolved gently and autoclave it at 121 °C for 15 min.

2.2.4 Mac Conkey agar

24 g of Mac Conkey agar were dissolved in 500 mL of distilled water and autoclave it at 121 °C for 15 min.

2.3.0 Serial Dilution Method

9.0 mL of distilled water was dispensed into test-tube sterilize by autoclaving at 121 ^{0}C for 15 min and allow it 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 it thoroughly and label 10^{-1} and introduced into the second test-tube. It is repeated up to 10^{-5} for each sample.

1.0 mL was also pipette from each test-tube and place in petri-dish 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 incubated at 30 ^{0}C for 72 h. The plates were placed in colony counter and recorded.

2.4 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.

2.5.1 Total and viable plate counts

The plate count method is most frequently employed for the enumeration of bacterial cells in a given sample. The plate count can be performed by using the pour plate method. In these methods overcrowding of colonies on plate should be avoided as this leads to inaccuracies in the count. Normally, plates with count ranging from 30 to 300 colonies are considered for population estimation. Therefore select a dilution which yields fewer than 300 colonies per plate since with colonial concentrations exceeding this count will usually be depressed to an unknown degree by overcrowding and micro antagonism.

2.5.2 Enumeration of micro organisms

To determine the number of microorganism in the fish sample is very important, in determined the safety of the fish product depending on knowing the level of microorganism present in particular fish product. Sample are taken for total number of living and dead cells, or by estimation of the number of living organism per mL this technique is used to assess the quality of the sample.

2.6. Bacteria Gram Stain Reaction

2.6.1 How to Gram Stain

Gram staining is a quick procedure used to look for the presence of bacteria in tissue samples and to characterize bacteria as Gram-positive or Gram-negative, based on the chemical and physical properties of their cell walls.

The Gram stain is always been done as the first step in diagnosis of a bacterial infection.

2.6.2 Steps to be followed in Gram Staining

Add 1-2 drops of the tissue sample onto a glass slide

↓

Heats fix the smear, by quickly passing it two to three times through a flame

↓

Flood the smear with crystal violet / gently rinse off the crystal violet with tap water

↓

Flood the smear with iodine / gently rinse off the iodine with tap water

↓

Decolorize by adding alcohol or acetone to the smear while holding the slide at an angle to allow the declouriser to drain

↓

Gently rinse off excess decolorized with tap water

↓

Flood the smear with safranin counter stain / gently rinse off excess safranin with tap water

↓

Drain slide and allow it to air dry

↓

Examine the slide under the light microscope

Fig 1: Flow diagram of gram staining bacteria.

2.6.4 Test for Gram-positive and Gram-negative Bacterial Identification

Gram Staining Procedure

In this process most bacteria have one of these two types of cell walls; we can use this difference as a feature that can be identified using the Gram stain. The Gram stain is a differential stain that uses two dyes to differentiate between the two basic bacterial cell wall types. The inoculate bacterial sample was taking from the skin and gill which was been incubated.

First a bacterial smear must be heat fixed to a microscope slide. A smear is a sample of bacteria suspended in a small amount of water on a slide. That sample is

then dried using heat. The heat kills the bacteria and attaches the sample to the slide so that it does not easily wash away.

The Gram staining procedure goes as follows:

- Flood the slide with Crystal Violet (the primary stain).
- After 1 minute, rinse the slide with water.
- Flood the slide with Iodine (Iodine is a mordant that binds with Crystal violet and is then unable to exit the Gram positive peptidoglycan cell wall.)
- After 1 minute, rinse the slide with water.
- Flood the slide with Acetone Alcohol. (Alcohol is a decolorizer that will remove the stain from the Gram-negative cells.)
- After 10 or 15 seconds, then rinse the slide with tap water. (Do not leave the decolorized for too long or it may remove stain from the Gram-positive cells as well.)
- Flood slide with Safrinin (the counterstain or additional stain for microscope specimen).
- After 1 minute, rinse the slide with water.
- Gently blot the slide dry. It is now ready to be viewed under oil immersion (1000x TM) with a bright-field compound microscope.

After this staining procedure, the Gram positive (+) cells will appear purple, having retained the primary stain. The Gram negative (–) cells will appear pink; having retained the counter stain after the primary stain was removed by the decolorizer.

2.6.5 Indole test:

Indole test is employed to determine the ability of isolate to break down the amino acid.

Material: test tube contains 1% peptone water, bacterial culture, kovac's reagent.

Inoculate the peptone water with the bacterial culture.

Incubate for 48 hours at 37^{oc}. Add 0.5ml of kovac's reagent and shake gently.

Presence of red color indicates indole (positive).

Absence of red color indicates not indole (negative).

2.6.6 Citrate Utilization test.

Tests for the ability of bacteria to convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle) and also help in identification of some members of the family Enterobacteriaceae. In this media, citrate is the only carbon source available to the bacteria. The media will turn a bright blue as a result of an increase in the pH of the media. To inoculate this slant, use the transfer loop. Inoculate the citrate agar plate with organism and inoculate at 30^{0c} for 72 hours for organism to grow.

2.6.8.1 MR-VP (Methyl red-Vogues Proskauer)

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

These tests are performed by inoculating a single tube of MR-VP media with a transfer loop and then allowing the culture to grow for 3-5 days. After the culture is grown, about half of the culture is transferred to a clean tube. One tube of culture will be used to conduct the MR test, the second tube serves as the VP test.

MR (Methyl red) test:

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

2.6.8.2 VP (Vogues Proskauer) test:

First alpha-napthol (also called Barritt's reagent A) and then potassium hydroxide (also called Barritt's reagent B) are added to the VP tube. The culture should be allowed to sit for about 15 minutes for color development to occur. If acetoin was produced then the culture turns a red color (positive result); if acetoin was not produced then the culture appears yellowish to copper in color (a negative result).

2.6. 9 Catalase:

This test is can be used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can

occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase will breaks H_2O_2 down into water and O_2. To perform the catalase test simply smear a small amount of the test organism onto the lid of a Petri plate/culture dish. Then add a drop of hydrogen peroxide to the smear. If bubbles become visible (these would be the O_2 bubbling up) then the test is positive and you can conclude that the organism makes catalase. A lack of bubbles indicates the absence of catalase. Because most aerobic organism make catalase while anaerobic organism cannot.

Equation.

H_2O_2 + Organism $\xrightarrow{\text{catalase}}$ $H_2O + O_2$

Smear small amount of test organism onto slide.

Then add a drop of hydrogen peroxide to the smear and watch for the bubble

Presence of gas bubbling up is positive

Absence of gas bubbling up is negative.

2.6.10 Oxidase test

To perform this test simply swab some of your test culture into one of the boxes on an oxidase dry slide. If a color change to purple or blue is evident at 30 sec-1 minute then the result is positive. It is important that the test is read by one minute to ensure accurate results (avoid false negatives and false positives). If it remain unchanged is negative.

2.6.11 Coagulase test

This test use to differentiate positive from negative bacterial in the presence of coagulation. The enzyme coagulase clots plasma by converting fibrinogen to fibrin.

Procedure

• A drop of physiological saline was place on each clean slide.

• Emulsify a colony of the test organism with wire loop on each of the drops to make thick suspensions.

• Add a drop of plasma or serum to the suspensions and mix gently. Look for clumping of organism within 10 seconds

• Presence of agglutination give positive.

• Absence of agglutination give negative.

2.6.12 Motility test

Some bacterial genera can move actively from one place to another; others that are not endowed with the requisite locomotory structure such as flagella cannot move. The ability to test this is important in their identification as it helps to differentiate mobile from non mobile organisms. The determination of motility among bacterial species can be demonstrated by hanging drop or stab culture in motility medium. A positive motility test is indicative of the presence of locomotory organ in the test isolate.

3.1 Microbiological Load of Catfish sample from skin and gills

Table 1 shows the result for the control experiment. Table 2 and 3 showed the microbial loads, microscopic and biochemical characteristics of the bacterial associate with catfish in three market locations in minna. The total coliform unit count (cfu/ml) of fish sample in Kure market is 1.64×10^3 cfu/mL and 7.2×10^4 cfu/mL getting from skin and gills. The total coliform 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. (Daniel *et al.,* 2016) The total coliform unit counts in Bosso market were 5.6×10^4cfu/ml and 4.0×10^5 cfu/ml getting from skin and gills. The total number of fungi colony count found in catfish sample from both skin and gills found in Kure market were 2.0×10^3cfu/ml and 1.0×10^2cfu/ml. while fungi colony found in Mobil market were 0.1×10^4 cfu/ml and 0.7×10^2 cfu/ml from both skin and gills. And fungi colony found in Bosso markets were 1.3×10^2 cfu/ml and 0.5×10^1 cfu/ml from both skin and gills respectively. In summary therefore, Table 4 shows the bacterial identified from isolate were *Escherichja coli, Staphylococcus spp, Streptococcus spp, Bacillus, Shigella, Enterobacter spp and Pseudomonas.* While Table .5 shows the fungi species isolated namely; were *Aspergillus spp* and *Rhizopus*. These are summarized on table 5.

Table 1: Microbial counts of fish samples from three various ponds and Control

Location Of Ponds	Part isolated from	Coliform count (cfu/ml)	Standard Organization of Nigeria, AL (4×10^2 cfu/ml)
Bosso pond	Skin	3.8×10^2	Satisfactory
	Gills	3.0×10^1	Satisfactory
Kure pond	Skin	2.4×10^2	Satisfactory
	Gills	2.6×10^2	Satisfactory
Mobil pond	Skin	4.6×10^3	Marginal
`	Gills	3.2×10^1	Satisfactory

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

Table 2: Microbial count of fresh catfish (*Clarias gariepinus*) obtained from three different locations.

S/No.	Location (SON), AL Of market (4×10^2 cfu/ml)	Part of fish	Coliform count (cfu/ml)	Fungi Count (cfu/ml)
1.	Bosso Market Marginal	Skin	5.6×10^5	1.3×10^2
2.		Gills	4.0×10^5	0.5×10^1 Marginal
3.	Kure Market Unsatisfactory	Skin	1.64×10^3	2.0×10^3
4.		Gills	7.2×10^4	1.0×10^2 Unsatisfactory
5.	Mobil Market Unsatisfactory	Skin	6.0×10^6	0.7×10^4
6.	Marginal	Gills	4.8×10^4	0.7×10^2

Key; Mkt = market. Cfu/ml = colony forming unit per meal

Table 3: CHARACTERIZASATION AND IDENTIFICATION OF BACTERIA ISOLATED

MCA	Location of market	part isolated from	Cells shape	colonial morphology Elevation	Gram form	Motility	Biochemical test carried MR	VP	Indole	Citrate	Catalase	Coagulase	micro-organism Reaction
$BOSMKT_{MN}$	Skin	Pink	Rods	Convex	circular	-	-	+	-	+	-	+	-	Bacilli spp
	Gills	White	Rods	Convex	Irregular	+	+	+	-	+	+	+	+	
$KURMKT_{MN}$	Skin	Pink	Cocci	Convex	spindle	+	+	-	+	+	-	+	+	Staphylococcus
	Gills	Pink	Rods	Convex	spindle	+	+	+	-	+	+	+	+	
$MOBMKT_{MN}$	Skin	Pink	Cocci	Convex	circular	-	-	-	+	-	-	-	-	
	Gills	White	Rods	Convex	circular	+	+	+	-	+	+	+	+	
BOSPOND	Skin	Green	Rods	Spherical		-	+	+	-	+	-	-	-	
	Gills	Pink	Cocci	Convex	spindle	+	-	-	+	-	-	+	+	
KURPOND	Skin	Green	Cocci	Convex	spindle	+	-	-	+	-	+	+	+	

		Gills		Pink		Rods		Convex spindle		+		+		
-		+		-		-		+		+				
MOBPOND		Skin		Pink		Rods		Convex spindle		+		-		
-		+		-		-		+		-				
		Gills		Pink		Rods		Convex spindle		+		-		
-		+		-		-		+		+				

Key; $BOSMKT_{MN}$ Bosso market Minna Niger State, $KURMKT_{MN}$ Kure market Minna Niger State, $MOBMKT_{MN}$ Mobil market Minna Nigger State, MCA Microscopic appearance

Table 4: Summary of the entire microorganism identified from the skin and gills of catfish

Location	Part of the fish	Bacterial	Fungi
Bosso market	Skin	*Escherichia coli*	-
	Gills	*Staphylococcus spp*	*Aspergillus niger*
	Skin	*Streptococcus spp*	*Rhizopus spp*
	Gills	-	
Kure market	Skin	*Pseudomonas aeniginosa*	
	Gills	*Escherichia coli*	*Aspergillus niger*
	Gills	*Staphylococcus spp*	*Aspergillus niger*
	Gills	*Bacillus subtilis*	-
Mobil market	Skin	*Escherichia coli*	*Rhizopus spp*
	Gills	*Staphylococcus aureus*	*Aspergillus flavus*
Bosso pond	Gills	*Enterobacter spp*	
	Skin	*Pseudomonas spp*	-
Mobil pond	Skin	*Streptococcus spp*	*Rhizopus spp*
	Gills	*Shigella spp*	

Kure pond -	Skin	*Escherichia*	*coli*
	Gills	*Staphylococcus*	*spp*
	Aspergillus niger		

Table 5: Morphological Characteristics and Identification of Mould Isolated in Catfish

Part of the Fish	Colour of Aerial Hyphae	Colour of Substrate Hyphae	Nature of Hyphae	Shape and kind of Asexual Sporangiophore	Presence of Special Structure or Conidiophores	Appearance of Sporehead	Characteristics Spore	Probable Organism
Gills	Whitish black	Brown	Septatate Haphae	Oval greenish foot cell long erect	Swell into			*Aspergillus*
					Conidia	Present	Septate conidia	Multi-nucleate *spp*
Skin	Dark green	Purplish red	Septed	Brown	Absent	Micro-conidia	Short-columer	*Rhizopore*

Discussion

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 *Escherichja coli, Staphylococcus spp, Streptococcus spp, Bacillus, Shigella, Enterobacter spp and Pseudomonas* and fungi species were *Aspergillus spp* and *Rhizopus*. 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,* (2006) 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.

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, (Adam and Moss, 2000). 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.

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 conterminers, such species are *Escherichja coli, Staphylococcus spp, Streptococcus spp, Bacillus, Shigella, Enterobacter spp* and. 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.

Recommendation

The following recommendation will help to foster in fish farming and deterioration of fish and its products. The proper sanitation should be implemented in the fresh fish market in order to maintain the hygienic environment. Awareness campaign need to be conducted at fish selling location on mode of fish handing to reduce contamination. Further studies need to be carried out in order to ascertain the point of entry of contaminers with a view of curtailing it.

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