# Bacterial load, composition and succession in the African catfish, *Clarias gariepinus* (Burchell, 1822) held at ambient temperatures.

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**Abstract:** Microorganisms, especially bacteria are the major causes of spoilage in fresh fish. In the coastal areas of developing tropical countries where ambient temperatures are usually high and access to modern preservation and processing technologies is low, fish is often left under ambient conditions for long periods after capture, resulting in quality deterioration and spoilage with attendant increases in post harvest losses. This study was carried out to identify, characterize and estimate the number of colony forming units (CFU) of microorganisms associated with freshly slaughtered African catfish, Clarias gariepinus held at ambient temperatures. Microorganisms were isolated from the flesh, gills, guts, mouth and skin of fish samples at successive 6-hour intervals post-slaughter for 42 hours when the fish were adjudged spoilt by a 5-member trained assessment panel. Determination of viable bacterial count was carried out by introducing aliquots of three dilutions of samples into nutrient agar plates, incubating at 37°C for 24hrs and counting the number of CFU. Microorganisms were thereafter identified using colonial and morphological characteristics and biochemical tests. Organoleptic assessment of fish samples were carried out by a 5-man trained panel. Results showed that a total of eleven microorganisms of pathogenic and/or spoilage importance were isolated at various intervals from fish samples, including Acinetobacter spp, Bacillus subtilis, B. megaterium, Citrobacter Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Proteus mirabilis, Pseudomonas lundensis and Staphylococcus aureus. Generally the number of CFU from various parts of fish increased significantly (P < 0.05) as time interval increased.

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Keywords: Clarias gariepinus, microbial load, spoilage, postharvest losses, food safety, preservation.

#### Introduction

Clarias gariepinus, commonly called Clarias belongs to the family Clariidae, and the group of prominent cultivable Clarias species found in Nigeria (Cowx, 1992; Olaosebikan and Raji, 1998). C. gariepinus is a valuable food fish with high aquaculture and nutritional importance. Besides its high quality flesh, distinctive taste and texture, Clarias commands good market price possibly due to its relatively low fat and absence of intramuscular spines compared to other common cultivable species (Eyo, 2001). Fish is a major source of nutrients for humans, providing a significant portion of the protein intake in the diets of a large proportion of people in developing countries where it represents one of the cheapest sources of animal protein. However, freshly harvested fish is highly perishable; and depending on harvesting techniques and handling, may deteriorate and spoil within six hours of landing (Narain and Nunes, 2007). Agbon et. al., (2002) and Saliu (2008) reported that fish spoilage in Nigeria is influenced to a large extent by high ambient temperatures, considerable distances of landing ports to points of utilization and poor as well as inadequate infrastructures for post-harvest processing and distribution. Apart from the high perishability of fish, consumer safety is an issue to be considered because fish is a good medium for rapid bacteria multiplication particularly when processed under unsanitary conditions. Fish is processed mainly by smoke-drying in Nigeria, however, smoking may not commence immediately after capture as fresh fish is usually left at ambient temperatures where bacterial proliferation is encouraged. Shewan (1977) and Austin (2002) observed that microorganisms associated with freshly caught fish are principally a function of the environment where it is caught. According to Lima dos Santos (1978) tropical freshwater fish have a microbial flora comprising 54% gram negative and 43% gram positive bacteria; while the flora of tropical marine fish species are 60% gram negative and 37% gram positive. Adebona (1981) reported a range of microbial load of 10<sup>2</sup> - 10<sup>3</sup> CFU/cm<sup>2</sup> on the skin of

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fresh Chrysichthys species caught in Nigerian waters, while Al-Harbi and Uddin (2008) observed levels ranging from  $4.3 \pm 2.9 \times 10^6$  to  $1.6 \pm 3.9 \times 10^7$  cfu/g in gills filaments and  $8.7 \pm 4.1 \times 10^9$  to  $5.4 \pm 3.2 \times 10^{10}$ cfu/g in intestines of the common carp, Cyprinus carpio cultured in ponds in Saudi Arabia. Generally, microbial load increases on freshly caught fish where appropriate preservation techniques are not employed immediately after catch. As the natural defenses of fish break down as a result of death, available nutrients are used by microorganisms to sustain their life processes and support rapid multiplication. With an increase in bacterial flora and load, decomposition of the fish is rapid. This study was undertaken to assess microbial proliferation in various parts of freshly slaughtered Clarias gariepinus and is indicative of spoilage pattern in the fish, microorganisms responsible for spoilage in this species and possible safety concerns of consumers of this important fish.

# Materials and Methods Sample Collection

A total of eight C. gariepinus samples of average weight  $800 \pm 10$ g were collected from the reservoir of the University of Agriculture, Abeokuta (UNAAB), Ogun State in September 2008. They were transported live in plastic kegs to the laboratory of the Department of Microbiology, (UNAAB).

## **Preparation of Sample**

Known weights of gill, mouth, gut, flesh and skin samples from experimental fish were aseptically dropped into 10ml sterile distilled water in order to release microorganisms on them. An aliquot of fish sample solution (1ml) was aseptically pipetted into 9ml sterile distilled water and mixed thoroughly to give 10ml of 10<sup>2</sup> dilution of the sample solution. The above procedure was repeated to obtain dilutions of 10<sup>3</sup> to  $10^{12}$ .

## **Isolation of Microorganisms from Fish samples**

Aliquots of 1ml fish samples were taken using a sterile syringe and cultured in Nutrient agar using the pour plate method. The plate was swirled to evenly distribute the agar and organisms; then allowed to stand for several minutes to cool. The plates were incubated at 37°C for 18 – 24 hours. Further su b culturing was carried out to obtain pure colonies. The pure culture was of the organisms were inoculated on nutrient agar slants and incubation was at 37°C for 24hours.

## **Total Viable Bacterial Count**

Aliquots of 0.5ml of three dilution factors were introduced into three Petri dishes each. Molten nutrient agar at about 45  $^{\circ}$  C was added and then mixed thoroughly and allowed to set. The set agar was later

incubated at 37°C for 24hrs after which plates were examined and the number of colony forming units (CFU) per plate counted.

## **Identification of Microorganisms**

Characterization of the organisms was based on colonial, morphological and biochemical characteristics of colonies (Table 2). Macroscopic examination of surface colonies on nutrient agar medium was used to determine the colour, edge, elevation, surface, shape and arrangement of microorganisms. Morphological characteristics were studied under the oil lens immersion microscope after Gram-staining.

#### **Biochemical Tests**

Biochemical tests carried out on the bacterial isolates were Catalase test, Coagulase test, Motility test, Indole production test, Citrate utilization test, Urease test and Sugar Fermentation tests (Table .3).

#### **Results**

Results of microbial load on the gills, gut, skin, mouth parts and flesh of freshly slaughtered C. gariepinus at 6-hour intervals (Figure 1) showed that microbial load on all the parts evaluated increased progressively from 0-42hrs post slaughter, though the rate of microbial proliferation varied within the period of study. Highest microbial load was found on the mouth, gut, gills, flesh and skin in that order. The microbial flora isolated 6-hourly from 0-42 hours and their significance revealed eleven bacterial species and they were identified (Table 1). Among the eleven isolates, dominant bacterial species were Staphylococcus aureus and Klebsiella pneumoniae.

## Discussion

The trend of microbial proliferation was similar in all parts of C. gariepinus evaluated, with a distinct lag phase of little or no bacterial proliferation between 0 and 6hrs, followed by a more rapid bacterial proliferation period or log phase between 6 and 36hrs, then a stationary phase from 36 - 42hours postslaughter in line with the findings of Al- Bulushi et. al. (2007). The relatively lower microbial load in fish flesh at 0, 6 and 12hrs of sampling confirmed the 'sterility' of the flesh during the lag phase as biochemical processes of rigor mortis within fish conferred some level of preservation on it until rigor was resolved with increased microbial proliferation encouraged by the build-up of nutrients post-rigor in line with the findings of Austin (2006). The high microbial population in the gills at these hours corroborates the findings of Al-Harbi and Uddin (2003). As time progressed postslaughter, microbial load increased in all parts of the fish evaluated. This is probably due to the rapid

multiplication of microorganisms and their migration to all parts of fish that were initially 'sterile'. The fish were considered fit for consumption up till the 24hr post-slaughter based on their microbial load as suggested by the International Commission on Microbiological Specification for foods, ICMSF (1986) Organisms isolated included Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Escherichia coli. Bacillus subtilis. Bacillus megaterium, Staphylococcus Citrobacter freundii, aureus, Pseudomonas lundensis, Proteus mirabilis Acinetobacter species. Klebsiella pneumoniae was found in all parts of fish evaluated. This confirms the ubiquitous nature and high medical importance of the Klebsiella group as reported by Obiamiwe and Berkowitz (2006). Organisms isolated comprised six spoilage organisms (Bacillus subtilis, mirabilis, Citrobacter freundii, Acinetobacter species, Pseudomonas lundensis, and Bacillus megaterium), three pathogenic organisms (Staphylococcus aureus, Escherichia coli, and Enterobacter cloacae) and two opportunistic pathogens (Klebsiella pneumoniae and Klebsiella oxytoca). Some of the organisms are significant as pathogens and also as spoilage agents. Proteus mirabilis, a spoilage agent which sometimes exist as an opportunistic pathogen under suitable conditions. *Pseudomonas species* are pathogens; however some of them are opportunistic pathogens, while four of the species (*Pseudomonas mudicolens*, *P. taetrolens*, *P. fragi*, and *P. lundensis*) are spoilage organisms. According to Gennari and Dragotto (1992), *Pseudomonas fragi* causes spoilage in dairy products, *P. mudicolens* and *P. taetrolens* cause mustiness in eggs, while *P. lundensis* causes spoilage in fish, cheese, meat and milk products.

Fish farmers should avoid culturing fish with water contaminated with fecal matter of animal origin including humans, by monitoring water source for pond aquaculture and the application of proper filtration when using other culture enclosures e.g. concrete and wooden tanks common in developing countries. This precaution will not only prevent the residence of bacteria in such waters but also protect the culture medium from the opportunistic activities of these microorganisms on fish under culture. Since most microorganisms, particularly pathogenic contaminate fish through improper handling and sanitation practices during processing, fish processors need to be thoroughly educated on the need for the maintenance of complete hygienic conditions during fish handling and processing.

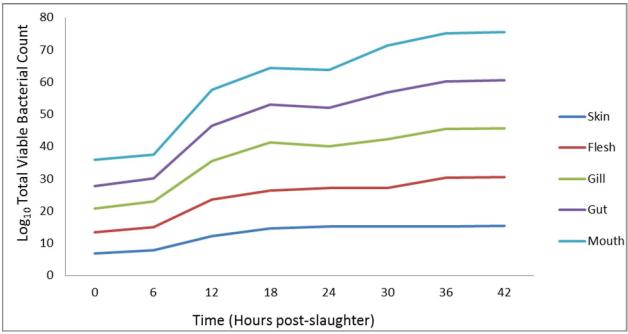


Figure 1: Bacterial load in different parts of *Clarias gariepinus* held at ambient temperatures

Table 1: Bacteria isolated six – hourly from different parts of *Clarias gariepinus* and their significance

Time	1: Bacteria isolated si	Gut	Gill	Flesh	Skin
(h)/Part	Mouth	Gut	GIII	riesn	Skin
of fish					
0 11811	Staphylococcus aureus	Citrobacter freundii(S)	Enterobacter	Citrobacter	Staphylococcus
•	(P)	carobacier freundings)	cloacae(P)	freundii(S)	aureus(P)
		Klebsiella	cioucuc(1)	Ji canan(s)	un cus(1)
	Enterobacter cloacae	pneumoniae(OP)	Bacillus	Staphylococcus	Klebsiella
	(P)		subtilis(S)	aureus(P)	pneumoniae(OP)
		Bacillus subtilis(S)	,	. ,	
	Klebsiella pneumoniae		Klebsiella	Klebsiella	Citrobacter
	(OP)	Staphylococcus	pneumoniae(OP)	pneumoniae(OP)	freundii(S)
		aureus(P)			
				Klebsiella oxytoc	
				Bacillus	
6	Enterobacter cloacae	Staphylococcus	Staphylococcus	megaterium(S)  Klebsiella	Staphylococcus
U	(P)	aureus(P)	aureus(P)	pneumoniae(OP)	aureus(P)
		uureus(I)	uur cus(1)	pheumoniac(O1)	uureus(1)
	Klebsiella pneumoniae		Klebsiella	Staphylococcus	
	OP)		pneumoniae(P)	aureus(P)	
	,		, ´		
	Staphylococcus				
	aureus(P)				
	D				
12	Proteus mirabilis (S)	VI-L-:-II-	C+ 1 1 -	C+ l	C4 I I
12	Staphylococcus	Klebsiella	Staphylococcus	Staphylococcus	Staphylococcus
	aureus(P)	pneumoniae(OP)	aureus(P)	aureus(P)	aureus(P)
	Acinetobacter	Enterobacter cloacae(P)	Klebsiella	Klebsiella oxytoca( <b>OP</b> )	Klebsiella
	species(S)	Emeroducier cioacac(1)	pneumoniae(OP)	nicosiciia oxyloca(OI)	pneumoniae(OP)
	<i>special</i> (2)		<i>p</i>	Citrobacter freundii(S)	F
	Enterobacter cloacae(P)		Klebsiella		Citrobacter freundii(S)
			oxytoca(OP)		
	Klebsiella				Proteus mirabilis(S)
	pneumoniae(OP)	****	***	~	~
18	Staphylococcus	Klebsiella	Klebsiella	Staphylococcus	Staphylococcus
	aureus(P)	pneumoniae(OP)	oxytoca(OP)	aureus(P)	aureus(P)
	Klebsiella pneumoniae	Staphylococcus	Klebsiella	Klebsiella	Klebsiella
	(OP)	aureus(P)	pneumoniae(OP)	pneumoniae(OP)	pneumoniae(OP)
	(01)	unicus(1)	pheamoniae(O1)	pheamoniac(OI)	pacamonac(O1)
		Enterobacter cloacae(P)	Enterobacter	Klebsiella oxytoca( <b>OP</b> )	Citrobacter freundii(S)
			cloacae(P)		J. J. Carrette (5)
				Citrobacter freundii(S)	
24	Staphylococcus	Citrobacter freundii(S)	Klebsiella	Enterobacter	Staphylococcus
	aureus(P)		pneumoniae(OP)	cloacae(P)	aureus(P)
		Staphylococcus			
	Klebsiella	aureus(P)	Klebsiella	Klebsiella	Escherichia coli(P)
	pneumoniae(OP)	Fortunal to 1	oxytoca(OP)	pneumoniae(OP)	V1-1-:-!
		Enterobacter cloacae(P)		Ct an hulo oo	Klebsiella
		Escherichia coli( <b>P</b> )		Staphylococcus aureus(P)	pneumoniae(OP)
		Escherichia con(1)		unicus(1)	Citrobacter freundii(S)
				Citrobacter freundii(S)	carobacier freuman(s)
30	Staphylococcus	Klebsiella	Klebsiella	Staphylococcus	Staphylococcus
	aureus(P)	pneumoniae(OP)	pneumoniae(OP)	aureus(P)	aureus(P)
	( )	1(~-)	1	, , ,	` ^
			Staphylococcus	Citrobacter freundii(S)	Citrobacter freundii(S)
			aureus(P)		
				Klebsiella	
			1	pneumoniae(OP)	ı

36	Staphylococcus aureus(P)	Klebsiella pneumoniae(OP)	Klebsiella pneumoniae( <b>OP</b> )	Citrobacter freundii(S)	Citrobacter freundii(S)	
	Enterobacter cloacae(P)	Citrobacter freundii(S)	Enterobacter cloacae(P)	Staphylococcus aureus(P)	Staphylococcus aureus(P)	
	Klebsiella pneumoniae(OP)		. ,	Bacillus subtilis(S)		
	Pseudomonas lundensis( <b>S</b> )					
42	Staphylococcus aureus(P)	Klebsiella pneumoniae( <b>OP</b> )	Klebsiella pneumoniae(OP)	Citrobacter freundii(S)	Citrobacter freundii(S)	
	Escherichia coli(P)	Klebsiella oxytoca( <b>OP</b> )		Staphylococcus aureus(P)	Staphylococcus aureus(P)	
					Klebsiella pneumoniae( <b>OP</b> )	

Table 2: Colonial and Morphological characteristics of bacteria isolated from different parts of *Clarias* 

gar	rie <sub>l</sub>	pın	us

Colour	Edge	Elevation	Surface	Shape	Arrangement	Isolated Organism			
	Undulated	01: 141	0 4	T 1	D 1: :	Klebsiella			
Cream	Undulated	Slightly raised	Smooth	Irregular	Rod in pairs & singles	pneumoniae			
Cicain	Undulated	Flat	Smooth	Irregular	Rod	Citrobacter			
Cream	Cildulated	1 Idi	Smooth	Integular	Rou	freundii			
Translucent		Raised	Mucoid	Irregular	Rod	Pseudomonas			
	Irregular					species.			
Cream	Undulated	Flat	Smooth	Irregular	Rod	Proteus			
						mirabilis			
~	Undulated	Slightly	Smooth	Irregular	Rod in pairs	Klebsiella			
Cream	** 11 . 1	raised	0 1		& singles	pneumoniae			
C	Undulated	Flat	Smooth	Irregular	Cocco-	Acinetobacter			
Cream	Undulated	Flat	Smooth	Irregular	bacillary Rod	species Proteus			
Cream	Ondulated	riat	Sillootii	inegulai	Kou	mirabilis			
Cream	Entire	Slightly	Wrinkled	Irregular	Rod in	Bacillus			
to	Little	raised	Willikica	meguiai	chains	subtilis			
brown									
	Entire	Raised	Glossy	Round	Cocci in	Staphylococcus			
Yellow			-		clusters	aureus			
Cream	Serrated	Raised	Mucoid	Irregular	Rod	Enterobacter			
					-	cloacae			
C	Serrated	Raised	Mucoid	Irregular	Rod	Enterobacter			
Cream	Undulated	C1: -1-41	Smooth	T 1	Rod in pairs	cloacae Klebsiella			
Cream	Undulated	Slightly raised	Smooth	Irregular	& singles	oxytoca			
Cicam	Undulated	Slightly	Smooth	Irregular	Rod in pairs	Klebsiella			
Cream	Chanacca	raised	Sillootii	meguiai	& singles	pneumoniae			
	Entire	Slightly	Wrinkled	Irregular	Strepto -	Bacillus			
Cream		raised			bacillus rod	megaterium			
	Undulated	Raised	Smooth	Irregular	Short rod	Escherichia			
Cream					in singles	coli			
a	Undulated	Slightly	Smooth	Irregular	Rod in pairs	Klebsiella			
Cream	P. C	raised	C1	D 1	& singles	pneumoniae			
Yellow	Entire	Raised	Glossy	Round	Cocci in clusters	Staphylococcus aureus			
1 cilow	Undulated	Flat	Smooth	Irregular	Rod	Citrobacter			
Cream	Ollunateu	1 lat	Sillouli	inegulai	Rou	freundii			
C1 <b>C</b> 41111	Undulated	Slightly	Smooth	Irregular	Rod in pairs	Klebsiella			
Cream		raised			& singles	pneumoniae			
Cream	Undulated	Slightly	Smooth	Irregular	Rod in pairs	Klebsiella.			
		raised			& singles	oxytoca			
Cream	Serrated	Raised	Mucoid		Rod	Enterobacter			
						cloacae			

Catalas Coagulas Motilit Indol Citrat Oxidas Glucos Mannito Capsulat Isolated Isolated m Stain a Organism Organis m Klebsiella Klebsiella pneumoniae Pneumonia Citrobacter Citrobacter freundii Freundii Pseudomonas Pseudomonas species. species. mirabilis Mirabilis Klehsiella Klehsiella pneumoniae Pneumonia Acinetobacter Acinetobacter Species Proteus Proteus mirabilis mirabilis subtilis subtilis Staphylococcu Staphylococcu aureus aureus Enterobacter Enterobacter cloacae cloacae Enterobacter Enterobacter cloacae cloacae Klebsiella Klebsiella oxytoca oxytoca Klehsiella Klehsiella pneumonia pneumoniae megaterium megaterium Escherichia Escherichia Klehsiella Klehsiella pneumonia pneumoniae Staphylococcu Staphylococcu aureus aureus Citrobacter freundii freundii Klehsiella Klehsiella pneumoniae pneumoniae oxytoca oxytoca Enterohacter Enterobacter cloacae cloacae

Table 3: Biochemical characteristics of bacteria isolated from different parts of Clarias gariepinus

# **Practical Application**

Microbial load and flora have implications on shelf-life and preservation of fish and its safety as food. Fish is a major source of food for large populations of people in most developing countries where they are often produced under unsanitary conditions thus exposing consumers to risks of poisoning with microbial toxins and also pathogens. Evaluation of microbial load and flora will improve knowledge of the microbiology of fish, risks involved in fish consumption and target organisms for preservation strategies.

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