ASSAY OF THE BACTERIAL FLORA IN THE GUT, GILL AND FLESH OF FRESH CATFISH (*CLARIAS GARIEPINUS*)

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ABSTRACT

This study was carried out to ascertain the bacterial flora in the gut, gill and flesh surfaces of fresh Clarias gariepinus purchased from four different markets located in Ota metropolis: Iyana-Iyesi, Oja-Ota, Oju-Ore and Sango-Ota. The fresh fish samples were randomly purchased from the markets and analysed using appropriate microbiological procedures. Bacterial isolates were identified based on colonial morphology, microscopy and biochemical tests. Analyses showed that the highest values of total bacteria counts (TBC) were 74.55 x 10^5 CFU/g in the gut, followed by that of the gill at 47.40 x 10^5 CFU/g and the flesh, 34.37 x 10^5 CFU/g; all from fresh C. gariepinus purchased from Oja-Ota market. There were five bacteria specie isolated from the gut, gill and flesh, namely Escherichia coli, Citrobacter spp., Salmonella spp., Shigella spp. and Proteus spp. The percentage occurrence of the bacteria species in the isolates indicated that Salmonella spp. had 36.4%, E. coli 27.3%, Citrobacter spp. 9.1%, Shigella spp. 18.1%, and Proteus spp. 9.1%. As a result of the presence of these pathogens in the fish; adoption of good fish culture standards, water quality and feeds, hygienic transportation and proper handling of fish by farmers and at the retail level by the vendors is highly recommended.

Keywords: Fresh catfish, Gills, Flesh, Gut, Bacterial isolates

INTRODUCTION

Fish is a major source of proteins of high digestibility and are rich source of lysine and sulphur containing amino acids (Cho and Kim, 2011). In Africa, over 17.5% of the animal protein comes from fish, while in Nigeria fish constitutes 40% of the animal protein intake of the people (Grema *et al*., 2011). The catfish, (*Clarias gariepinus* Burchell 1822, Siluriformes: Clariidae) is a very important freshwater fish in Nigeria (Idodo-Umeh, 2003) and enjoys wide acceptability in most part of the country because of its unique taste, flavor and texture. Onyia *et al*. (2014) reported that fish is a major source of animal protein and an essential food item in the diet of Nigerians because it is relatively cheaper than meat. Fish protein provides excellent amino acids especially, the three that are lacking in protein of plant origin namely lysine, methionine and tryptophan. It also contains mineral elements such as zinc, phosphorus, iron and calcium (Famurewa *et al.,* 2017). In addition, fish is a good source of riboflavin, vitamins A and D (NIH, 2022).

Fresh fish is susceptible to spoilage as soon as it is caught and deterioration progresses until the fish is entirely destroyed. The limited shelf life of dead fish in Nigeria is 16 – 20 hours in Southern part and 20 – 36 hours in the Northern part are basically due to the biochemical changes after death and high ambient temperature (Aberoumand, 2010; Omoruyi *et al*., 2017).

Fish spoilage depends on hygienic conditions, storage temperature, acidity and the structure of the muscular tissue (Singh *et al*., 2011). Chemical breakdown of protein content, fat content (agent of rancidity and off-flavour) and the water content/water activity contribute to quick spoilage of fish (Daramola *et al*., 2007). However, the extent of fish spoilage depends on the processing techniques, the type of fish being processed, weather and mode of storage during transportation (Ghaly *et al*., 2010).

After fish is caught and died, the immune system collapses and bacteria can freely proliferate on the skin surface, gill, gut and the stomach. About one-third of the world's food production is lost annually as a result of microbial spoilage (Omojowo *et al*., 2010). Microorganisms inhibit nearly every niche of the earth and our food is no exception (Hibbing *et al*., 2010). Microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods (Lund *et al*., 2000) and the load increases with increasing temperature; resulting in rapid fish spoilage.

Pathogenic bacteria associated with fish can be categorized into three general groups: Bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*). Enteric bacteria (non-indigenous bacteria) that are present due to faecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli, Staphylococcus aureus*); and bacteria contamination during processing and preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp.) (Lyhs, 2009).

The consumption of *C. gariepinus* is on the increase in both rural and urban centers of Nigeria (Emikpe *et al*., 2011). However, previous studies have reported bacteria load in catfish (Andy *et al.,* 201; Effiong and Isaac, 2019; Afolabi *et al*., 2020). Therefore, this is a further contribution to the knowledge of bacterial flora of catfish, arising from abiotic and biotic dynamics in their habitats due to climate change. These changes have been reported to affect the distribution and abundance of bacteria in fishes (Razak *et al*., 2019). Furthermore, the essence of tis study was to ascertain the biomass and species of the bacteria in catfish and create the awareness of the pathogens to would-be consumers. The present study was designed to investigate the total bacterial counts in the gut, gill and flesh of *C. gariepinus*, sourced from different markets in Sango-Ota metropolis, Ogun State, Nigeria.

MATERIALS AND METHODS

Study Site: Fresh *C. gariepinus* samples were purchased between April 4 – May 9, 2022 from four different markets in Ota, namely Iyana-Iyesi (M1), Oja-Ota (M2), Oju-Ore (M3) and Sango-Ota (M4); three catfishes were purchased from each market. The fish specimen, *C. gariepinus* was morphologically identified (Olaosebikan and Raji, 2004) and authenticated by a fish taxonomist and a voucher specimen (BUT-DBSM-2022-CG-001) kept in the departmental museum for referral purposes. Microbiological analysis was carried out at the Microbiology Laboratory of the Department of Biological Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

Sample Processing: All samples (live fresh catfish) obtained were stunned to death using the ice-slurry immersion method (Blessing *et al.,* 2010). Different parts of catfish such as the gut (X), gills (Y) and flesh (Z) were cut using sterile surgical blades. Samples were then separately crushed using a sterilized mortar and pestle.

Laboratory Analysis: Glass wares sterilization and media/agar (Nutrient, MacConkey, Eosin Methylene Blue and Salmonella-shigella) preparations were carried out following the standard methods (Cheesbrough, 2000). Thereafter, one gram of the catfish gut, gill and flesh were separately weighed from the crushed or macerated fish portion and homogenized with 9 ml of distilled water in a MacCartney bottle. Then, serial dilution of sample homogenates was prepared.

Incubation of culture plates was for 24 – 48 hours. Microbial counts and identification were carried out using standard procedure and

biochemical tests such as the Gram staining techniques, catalase test, Kligler iron agar test, Sulphide indole motility test, citrate utilization, coagulase test and Gram reaction (Baker *et al.,* 2000; Cheesbrough, 2000).

Bacterial isolates were characterized using routine microbiological procedures as described by Varghese and Joy (2014) after which they were identified using Bergey's Manual of *Systematic* Bacteriology (Bergey, 2001).

Data Analysis: Data collected on the bacterial load of catfishes from the different markets were analysed for their central tendencies using descriptive statistics. Results of the total bacterial counts (CFU/g) were presented in figure, while the presence or absence of the bacterial isolates in the different markers was tabulated.

RESULTS

The results in Figure 1 showed the total bacterial counts in the gut, gill and flesh of the fresh catfish samples obtained from four markets: Iyana-Iyesi (M1), Oja-Ota (M2), Oju-Ore (M3) and Sango-Ota (M4).

In Figure 1, at the Iyana-Iyesi market (M1), the total bacteria counts (TBC) were 73.80 x 10^5 CFU/g (gut), 46.11 x 10^5 CFU/g (gill) and 32.87 x 10^5 CFU/g (flesh).

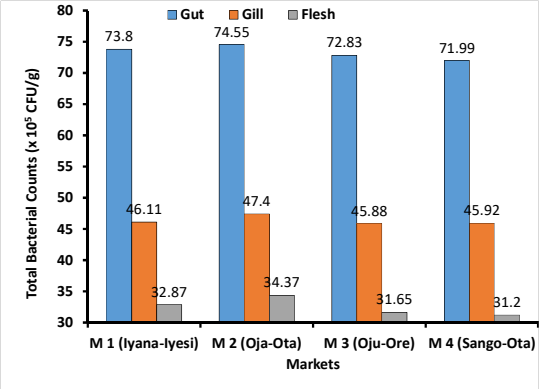

Figure 1: The total bacterial counts from the gut, gill and flesh of *Clarias gariepinus* sampled from four markets in Ota, Ogun State, Nigeria

The highest values of TBC were 74.55 x 10^5 CFU/g in the gut, followed by that of the gill at 47.40 x 10^5 CFU/g and the flesh, 34.37 x 10^5 CFU/g; all from fresh *C. gariepinus* purchased from Oja-Ota market (M2). For the fresh catfish sampled from Oju-Ore market (M3), the TBC value of 72.83 x 10^5 CFU/g was recorded in the gut, 45.88 x 10^5 CFU/g from the gill and 31.65 x 10^5 CFU/g from the flesh. Also, at Sango-Ota (M4), the TBC were 71.99 x 10^5 CFU/g, 45.92 x 10^5 CFU/g and 31.20 X 10^5 CFU/g in the gut, gill and flesh respectively.

Therefore, it was evident that the highest TBC in the gut, gill and flesh of the *C. gariepinus* samples purchased freshly from the four selected markets in Ota metropolis were from the main market, Oja-Ota (M2) followed by that of samples from Iyana-Iyesi, then Oju-Ore and Sango-Ota markets.

There were five bacteria species isolated and were identified based on colonial morphology, microscopy and biochemical tests, from the gut, gill and flesh (Table 1). These were namely the *E. coli*, *Citrobacter* spp., *Salmonella* spp., *Shigella* spp. and *Proteus* spp. The percentage occurrence of the bacteria species in the isolates indicated *Salmonella* spp. had 36.4%, *E. coli* 27.3%, *Citrobacter* spp. 9.1%, *Shigella* spp. 18.1%, and *Proteus* spp. 9.1%.

DISCUSSION

Microbial pathogens associated with fish can be transmitted to human that uses the fishes as source of food. Aquaculture products can harbor pathogenic bacteria which are part of the natural microflora (Muhammad *et al.*, 2020).

Meanwhile, most customers mostly assess the quality of fish by considering the appearance, smell, and palatability when cooked; hence, it is necessary to produce good quality and safe fresh fish free from harmful microbial load (Abidemi-Iromini *et al.*, 2011). A high population of bacteria in food indicates the general quality of the food and the degree of spoilage it might have undergone.

In this study, the biochemical test carried out on isolates from those fresh fish showed the presence of *Salmonella* species, *E. coli*, *Citrobacter* species, *Proteus* species and *Shigella* species.

Table 1: Biochemical identification of bacterial isolates from parts of the catfish

Sample	CC	GR	Shape	K	A	H_2S	G	IND	MOT	CIT	CAT	OXI	Identification
SSA M2X	Colorless with black center	-	Bacilli	+	+	+	-	+	-	+	+	+	*Salmonella* spp.
EMB M2X	Metallic sheen	-	Rod	-	+	+	-	-	+	+	+	+	*Escherichia coli*
MCA M3Z	Light pink	-	Rod	+	+	+	-	+	+	+	+	+	*Citrobacter* spp.
SSA M3X	Yellow	-	Rod	+	+	+	+	-	+	+	-	+	*Salmonella* spp.
EMB M2X	Metallic sheen	-	Rod	-	+	-	+	+	+	+	+	+	*Escherichia coli*
MCA M2X	Colorless with black center	-	Bacilli	+	+	+	-	+	-	-	+	-	*Salmonella* spp.
SSA M1Z	Colorless with black center	-	Bacilli	+	-	-	-	+	-	-	+	-	*Shigella* spp.
EMB M1Z	Metallic sheen	-	Rod	-	+	-	+	+	+	+	+	-	*Escherichia coli*
MCA M1X	Colorless with black center	-	Bacilli	+	-	-	-	+	-	-	+	-	*Shigella* spp.
SSA M1X	Yellow	-	Rod	+	+	+	-	-	+	+	-	-	*Salmonella* spp.
SSA M4X	Colorless with black center	-	Bacilli	+	-	+	+	+	-	+	+	-	*Proteus* spp.

Key: *CC = Colonial character, GR = Gram reaction, k = Lactose production, A = Glucose production, H_2S = Hydrogen sulphide, G = Gas, IND = Indole, MOT = Motility, CIT = Citrate, CAT = Catalase, OXI = oxidase, M = Markets: 1, 2, 3 and 4, X = Gut, Y = Gill, Z = Flesh, + = present, - = absent*

According to Edberg *et al.* (2000), the occurrence of *E. coli* is suggestive of faecal contamination of the water from which the fishes were reared. The isolation and identification of bacteria from fish and its environment is essential strategy to identify, prevent and combat disease causing pathogens (Kassa and Mitiku, 2021). Improper handling, poor hygiene and bad culturing or fish holding water medium have been reported to lead to the contamination of food and this might eventually affects the health of the consumers (Okonko *et al.*, 2008 a,b; Mir *et al.*, 2018). Meanwhile, Okonko *et al.* (2010) revealed that pathogens were present in the palm swabs of all the frozen seafood processors/handlers and water used by them. Between their palms and the water sampled, *Bacillus* spp., *Staphylococcus* spp., *Enterobacter* spp., *Flavobacterium* spp., *Micrococcus* spp., *Pseudomonas aeroginosa*, *Streptococcus faecalis* and *E. coli* were isolated. Therefore, inadvertently food processors may be sources of microbial inoculation, microbial food poison, food intoxication and food spoilage (Bankole *et al.*, 2009). Also, Taiwo *et al.* (2013) found in between the scales, skin and gut of the fish sample analysed organisms such as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Citrobacter* spp., *Streptococcus* spp., *Vibrio cholera*, *Proteus* spp. and *Staphylococcus* spp. Meanwhile, similar studies has been conducted on the microbial loads on different parts of fresh fish such as the scales, skin, gill and gut (Shinkafi and Ukwaja, 2010; Emikpe *et al*, 2011; Ajayi, 2012). In this study, the gut was found to contain more bacteria loads and species than in other parts of the fish samples and this corroborated the findings of Mandal *et al.* (2009) and Taiwo *et al.* (2013). In developing countries, contaminants such as bacteria, viruses, fungi, protozoa and helminths have been reported to be are responsible for food borne diseases such as cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, amoebiasis and poliomyelitis (Ramírez-Castillo *et al.*, 2015). This was in

agreement with the findings of Gram *et al*. (2000) that microbial spoilage could predispose consumers to health hazards resulting from food poisoning. *B. cereus* is known to cause enterotoxigenicity due to the production of enterotoxin and also known to cause *Staphylococcus* food poisoning which is a major food intoxication (Nwachukwu and Madubuko, 2010). Also, the high level of TBC in the fresh *C. gariepinus* in the main market, Oja-Ota, can be attributed to the poor hygiene or environment, large number of traders, human contacts with antecedent possibility of contamination by different activities around and the open, bare, dusty floor of the market. This was in line with Novoslavskij *et al*. (2016) who stated that fish take a large number of bacteria into their gut from water sediment and food. Similarly, it has been established that both fresh and brackish water fishes can harbour human pathogenic bacteria particularly of the coliform group (Cabral, 2010; Amuneke *et al*., 2020). Therefore, the presence of enteric bacteria in fish is seen as a sign of poor standards process of hygiene and sanitation (Daramola *et al*., 2020).

Conclusion: This research showed that pathogenic organisms are present in different parts of fresh fish. The microbial loads of the gut, gills and flesh of catfish purchased from different markets showed that the gut had the highest microbial load, while the microbial loads of flesh was low when compared to the microbial loads of gills. The diversity of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. The presence of this pathogens, though not at lethal levels showed there is the need for proper monitoring of the standards of fish and products in our markets. Fish ponds, rivers, lakes and different materials used for breeding catfish could be major source of contaminant. Fish feeds are known to contaminate fish pond-water which can contaminate the fish. To reduce such contamination, fish farmers should be educated more on the breeding and processing of catfish to ensure that fish pond water are properly treated and should be free of contaminants. Fish feed should be properly prepared and free of contaminants. All fish consumers should ensure that gills and gut are removed from the fish before cooking and proper washing of catfish flesh are done before cooking so as to reduce the microbial loads of fish. Also proper and thorough cooking of catfish should be done before consuming fish.

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