

Bacteriological Evaluation Of Tilapia Fish And Crab Bought From Rumuola Market, Rivers State, Nigeria

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Abstract

The bacteriological evaluation of Tilapia fishes and crabs bought at Rumuola market, Port Harcourt, Rivers State, Nigeria, was carried out. A total of thirty (30) samples each of tilapia fishes and crabs were obtained from Rumuola market. The intestines, gills and flesh of tilapia fishes and carapace meats of crab samples were analysed for total heterotrophic bacteria, total coliform and Salmonella species employing standard microbiological procedures replicated thrice. Results obtained from the study show that the highest total heterotrophic bacteria counts for fish and crab were 2.75×10^8 cfu/g and 2.29×10^8 cfu/g respectively; the highest total coliform counts for fish and crab were 2.47×10^8 cfu/g and 2.36×10^8 cfu/g respectively, while the highest Salmonella count were 2.71×10^8 cfu/g and 6.4×10^8 cfu/g respectively. All the counts were above the ICMSF recommended limit of 1×10^5 cfu/g for seafood which could be due to source contamination, improper handling and unhygienic environment. The study also revealed that Micrococcus spp. Proteus mirabilis, Corynebacterium spp. Bacillus spp. Enterobacter spp. Salmonellatyphi and Citrobacterspp, were isolated and identified. These have public health implication, meaning the proper cooking done during preparation to prevent cross contamination and eventual food intoxication. Generally, hygienic practices and storage condition are recommended for the marketers.

Keywords: Bacteria, Contamination, Crab, Market, Tilapia

Introduction

Fishing is a prehistoric practice dating back at least 40,000 year. Bekker-Nelson (2005) reported that since the 16th century, fishing vessels have been able to cross in pursuits of fish and since the 19th century it has been possible to use large vessels and in some cases process the fish on board. According to Adebayo-Tayo *et al.* (2012), fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, as well as, the environmental and feeding habits. Fish has become an important source of protein and other elements necessary for maintenance of healthy body (Adebayo-Tayo *et al.*, 2012). Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita intake of animal protein (FAO 2016). The agricultural sector in Nigeria engages up to 70% of employees and fish plays a significant role since it accounts for 50% of total animal protein consumption by the larger percentage of the population (FDF, 2009). They have significant role in nutrition, income, employment and foreign exchange earning of the country (Ikapeet *et al.*, 2018). Fish is the favorite source of animal protein compared to pork, poultry, beef, or mutton. People in the world depend on fish as a supplement of their food nutrients. Fish has become an increasingly important source of protein and other elements necessary for the maintenance of a healthy body (Emikpeet *et al.*, 2011). Fish and seafood constitute an important food component for a large section of the world's population (Emikpeet *et al.*, 2011). The nutritional characteristics of fish and fishery products are of vital interest to consumers. Today, even more people are turning to fish as a healthy alternative to red meat (Adebayo-Tayo *et al.*, 2012).

Crabs make up 20% of all marine crustaceans caught and farmed worldwide, with over 1.5 million tonnes being consumed annually (Onadeko *et al.*, 2015). Crabs mostly occur at the mouth of estuaries and along the course of many rivers (Fagbua *et al.*, 2013). They constitute a nuisance by damaging set nets in the water, feeds on fish, aquatic vegetation, mollusks, crustaceans, and annelids. Crabs are decapods crustaceans rich in sodium, potassium, and phosphorus with high amounts of iron, zinc, copper, and manganese. They also present high concentrations of vitamins A, C, B6, thiamine, and riboflavin and are considered a delicacy in several parts of the world (Vieria *et al.*, 2004). Fish and shellfish can also function as carriers of several microbial and other health hazards (Yagoub, 2009). Therefore, the maintenance of quality is of utmost importance in production and trade of fishery products. Environment quality, as well as the mode of collection and processing of products, may affect the quantity and diversity of microorganisms present on the surface of seafood and fishery products, which may increase microbial contamination (Ristoriet *et al.*, 2007). Detection of pathogenic bacteria in seafood is essential to ensure safe products for consumers, sustainable fish, and shellfish growing activities (Carvalho *et al.*, 2016). *Oreochromis niloticus* and *Brachyura* can serve as potential vectors for organisms that cause infections. Therefore, this work seeks to ascertain the bacteria associated with *Oreochromis niloticus* (tilapia fish) and *Brachyura* (crab) bought from Rumuola market.

Materials and methods

A total of thirty (30) samples each of tilapia fish and crab were obtained from Rumuola market. Each of the fish and crab sample were aseptically collected using sterile bags properly labeled according to standard microbiological procedures. The samples were then transported to the Biology/Microbiology 1 Laboratory, Captain Elechi Amadi Polytechnic, Rumuola, for microbiological analyses within 1 hour of collection in ice box. This was replicated thrice. The fish samples were prepared for bacteriological analysis as described by Yagoub (2009). The fish samples were rinsed with water to remove surface dirt and then the body surfaces were swabbed

with ethanol to remove external microorganisms. The skin, gills, and intestine were dissected out with the aid of sterile knife and forceps and separately macerated aseptically, using sterile mortar. One gramme (1g) of each homogenised fish part was suspended in 9ml of sterile normal saline. Thereafter, a ten-fold serial dilution up to 10^{-6} was carried out and employing spread plate technique, 0.1 ml of the suspension was inoculated onto freshly prepared Nutrient agar (NA), MacConkey Agar and Salmonella-Shigella (SS) Agar plates, for enumeration of total heterotrophic bacteria, total coliform and Salmonella respectively. The inoculated agar plates were then incubated at 37°C for 24 hours and characteristic colonies were counted and reported as colony forming unit per gram (cfu/g). Discrete bacterial colonies that grew on the respective media plates were subcultured using streaked plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure cultures. The identification of the isolated bacteria was done using cultural morphology, Gram staining and biochemical tests according to Holt *et al.* (1994) and Cheesborough (2006).

Results

The total heterotrophic bacteria counts obtained from gills, flesh and intestines of tilapia are presented in Table 1. The results revealed varying levels of microbial contamination of the fish samples. The counts from the gills ranged from 1.47×10^8 cfu/g to 2.75×10^8 cfu/g; counts from the flesh ranged from 1.73×10^8 cfu/g to 2.74×10^8 cfu/g and intestine counts ranged from 1.36×10^8 cfu/g to 2.69×10^8 cfu/g. The total coliform counts obtained from gills, flesh and intestines of tilapia are presented in Table 2. The counts from the gills ranged from 1.60×10^8 cfu/g to 2.44×10^8 cfu/g; counts from the flesh ranged from 1.13×10^8 cfu/g to 2.29×10^8 cfu/g and intestine counts ranged from 1.16×10^8 cfu/g to 2.47×10^8 cfu/g. The *Salmonella* counts obtained from gills, flesh and intestines of tilapia are presented in Table 3. The counts from the gills ranged from 2.05×10^8 cfu/g to 2.71×10^8 cfu/g; counts from the flesh ranged from 1.52×10^8 cfu/g to 1.90×10^8 cfu/g and intestine counts ranged from 1.88×10^8 cfu/g to 2.58×10^8 cfu/g. The results revealed varying levels of microbial contamination of the fish samples.

Table 1 Total Heterotrophic Bacterial Count obtained from *Tilapia* Fish

Sample	Counts Obtained (cfu/g)		
	Fish Parts		
	Gill	Flesh	Intestine
Fish 1 10^8	1.47×10^8	2.19×10^8	1.36 X
Fish 2 10^8	2.49×10^8	1.87×10^8	1.89 X
Fish 3 10^8	2.39×10^8	1.73×10^8	2.59 X
Fish 4 10^8	2.29×10^8	2.58×10^8	2.29 X
Fish 5 10^8	2.75×10^8	2.74×10^8	2.69 X

Table 2 Total Coliform Count obtained from *Tilapia* Fish

Sample	Counts Obtained (cfu/g)		
	Fish Parts		
	Gill	Flesh	Intestine
Fish 1 10^8	1.74×10^8	1.80×10^8	1.16 x
Fish 2	1.72×10^8	2.10×10^8	1.59 x

10 ⁸				
Fish 3	1.60 x 10 ⁸	1.13 x 10 ⁸	1.38	x
10 ⁸				
Fish 4	2.04 x 10 ⁸	2.25 x 10 ⁸	1.74	x
10 ⁸				
Fish 5	2.44 x 10 ⁸	2.29 x 10 ⁸	2.47	x
10 ⁸				

Table 3 Salmonella Count obtained from Tilapia Fish

Sample	Counts Obtained (cfu/g)			
	Gill	Fish Parts Flesh	Intestine	
Fish 1	2.71 x 10 ⁸	1.80 x 10 ⁸	2.51	x
10 ⁸				
Fish 2	2.21 x 10 ⁸	1.90 x 10 ⁸	2.30	x
10 ⁸				
Fish 3	2.05 x 10 ⁸	1.52 x 10 ⁸	2.58	x
10 ⁸				
Fish 4	2.59 x 10 ⁸	1.55 x 10 ⁸	2.57	x
10 ⁸				
Fish 5	2.58 x 10 ⁸	1.79 x 10 ⁸	1.88	x
10 ⁸				

The total heterotrophic bacteria, total coliform and salmonella counts obtained from water crabs gills are presented in Table 4.

The total heterotrophic bacteria counts shows that crab 5 had the highest count of 1.59 x 10⁸ cfu/g while crab 3 had the lowest count of 2.29 x 10⁸ cfu/g; for total coliform bacteria, crab 3 had the highest count of 1.53 x 10⁸ while crab 1 had the lowest count of 2.36 x 10⁸ cfu/g, and for Salmonella, crab 5 had the highest count of 6.4 x 10⁸ cfu/g while crab 3 had the lowest count of 1.24 x 10⁸ cfu/g.

Table 4 Bacterial counts obtained from crabs (Carapace Meats)

Sample	Counts Obtained (cfu/g)		
	THB	TCB	
Crab 1	2.05 x 10 ⁸	2.36 x 10 ⁸	1.71
10 ⁸			x
Crab 2	1.87 x 10 ⁸	1.92 x 10 ⁸	1.32
10 ⁸			x
Crab 3	2.29 x 10 ⁸	1.53 x 10 ⁸	1.24
10 ⁸			x
Crab 4	1.98 x 10 ⁸	2.04 x 10 ⁸	1.57
10 ⁸			x
Crab 5	1.59 x 10 ⁸	1.89 x 10 ⁸	6.4 x 10 ⁸

Key: THB total heterotrophic bacteria TCB total coliform bacteria

Discussion

Fish and shellfish do not only transmit diseases to man but are themselves subject to many food borne microbial infections and intoxications (Emikpe et al., 2011). This study attempt to assess the bacterial contents tilapia and water crabs collected from the Rumuola market.

The results revealed varying levels of microbial contamination of the fish samples. The counts from the gills ranged from 1.47×10^8 cfu/g to 2.75×10^8 cfu/g; counts from the flesh ranged from 1.73×10^8 cfu/g to 2.74×10^8 cfu/g and intestine counts ranged from 1.36×10^8 cfu/g to 2.69×10^8 cfu/g. The counts obtained were higher than the 4.7×10^6 cfu/g to 5.2×10^6 cfu/g (flesh), 5.5×10^6 to 6.0×10^6 cfu/g (gill) and 6.6×10^6 to 7.0×10^6 cfu/g (intestine) reported by Daminabo et al. (2021). The total bacterial counts obtained from this work were found to be higher than the specified standard limits of 1×10^5 cfu/g by ICMSF (1982). Ogbonna and Inana (2018) have earlier stated that the high bacteria counts could be attributed to poor water quality, organic loading of the aquatic environment, handling and transport of fish by vendors, marked temperature changes, poor storage condition, hypoxia or other stressful conditions.

The total coliform counts in this study for gills ranged from 1.60×10^8 cfu/g to 2.44×10^8 cfu/g; counts from the flesh ranged from 1.13×10^8 cfu/g to 2.29×10^8 cfu/g and intestine counts ranged from 1.16×10^8 cfu/g to 2.47×10^8 cfu/g. Daminabo *et al.* (2021) reported a total coliform counts ranging from 4.3×10^6 cfu/g to 5.0×10^6 cfu/g for gills, 3.3×10^6 cfu/g to 4.1×10^8 cfu/g for flesh and 4.7×10^6 cfu/g to 5.9×10^6 cfu/g for intestine, which were comparatively lower than the counts reported in this study. The study observes that handling, hygiene and storage conditions in the markets before purchase by consumers could be contributing factors. Al-Hind *et al.* (2011) have earlier reported that total coliform count range became higher as duration of storage increases.

The Salmonella counts from the gills ranged from 2.05×10^8 cfu/g to 2.71×10^8 cfu/g; counts from the flesh ranged from 1.52×10^8 cfu/g to 1.90×10^8 cfu/g and intestine counts ranged from 1.88×10^8 cfu/g to 2.58×10^8 cfu/g. In another study, Umana *et al.* (2017) had reported 4.7×10^3 cfu/g, 5.03×10^3 cfu/g and 0.00×10^3 for skin, gill and intestine respectively obtained from *Oreochromis niloticus* and 4.10×10^3 cfu/g, 0.00×10^3 cfu/g and 0.00×10^3 cfu/g for skin, gill and intestine obtained from tilapia guinensis. Some studies have also reported the high incidence of *Salmonella* in fish skin, gills and (Nwiyi and Onyebor, 2012), as well as muscle (El-Olemy *et al.*, 2014). According to ICMSF (1986), *Salmonella* should not be found in sea food products; therefore, this level of contamination is unacceptable. However, in the present study, high numbers of *Salmonella* was isolated from fresh tilapia fishes. The fishes are cooked before consumption and therefore, can cause negligible health risks to the consumers except for cross contamination in the kitchen (Huss, 1994). Umana *et al.* (2017) added that the high contamination level observed in studies like this simply indicates gross contamination during the period of exposure by the fishmongers in the open markets. Cross-contamination associated with raw and processed food through contact surfaces is considered a potentially dangerous event, and some authors have reported the presence of *Salmonella* on stainless steel surfaces (Wang *et al.*, 2015). This study reported that all of the fish samples along with the materials did not meet the standard levels suggested by ICMSF, despite the aseptic and proper hygienic conditions properly maintained throughout all including catching, landing, transportation, processing, handling, and preservation. According to the rule of International Association of Microbiology Society, fresh and frozen fish should possess neither *Vibriospp.* nor *Salmonellaspp.* (Sanjee and Karim, 2016).

The total heterotrophic bacteria counts shows that the highest count was 1.59×10^8 cfu/g while the lowest count was 2.29×10^8 cfu/g. The counts obtained were higher than the 1.6×10^2 - 2.36×10^3 cfu/g reported by Oramadike and Kolade (2015), who also stated that the microbial load could be as a result of anthropogenic activities around the river where the crabs were caught, in addition to the handling by the fishermen and the marketers. The highest and the lowest total coliform counts

were 1.53×10^8 and 2.36×10^8 cfu/g respectively. These were slightly higher than the range, 5.30×10^7 – 8.40×10^7 cfu/g reported by Adebayo-Tayo and Okpo (2010) in their study.

Salmonella counts show that the highest count was 6.4×10^8 cfu/g, while the lowest count obtained was 1.24×10^8 cfu/g, which were slightly higher than the ranges, 3.95×10^4 cfu/g - 16.76×10^4 cfu/g and 6.20 – 6.80×10^7 cfu/g reported by Adebayo-Tayo and Okpo (2010) and Karim *et al.* (2015) respectively. The microbial loads from the crab analysis were higher than the recommended limits, 1×10^5 cfu/g for bacteria and 1×10^2 cfu/g for coliforms by ICMSF (1986) and USFDA (1991). Adebayo-Tayo and Okpo (2010) stated that this high level of microbial loads could be as a result of human activities such as disposal of faecal matters and sewage discharge in the rivers where the shellfish are harvested. The study revealed that *Micrococcus* spp., *Proteus mirabilis*, *Corynebacterium* spp., *Bacillus* spp., *Enterobacter* spp., *Salmonella typhi* and *Citrobacter* spp., were isolated and identified. *Salmonella* spp., *Micrococcus* spp., *Proteus mirabilis*, *Corynebacterium* spp., *Bacillus* spp., *Enterobacter* spp., *Citrobacter* spp., *Vibrio* spp. and *Shigella* spp. Karim *et al.* (2015) reported *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. Daminabo *et al.* (2021) also reported *Salmonella* spp., *Vibrio* spp., *Bacillus* spp., *Shigella* spp., *Enterobacter* spp. and *Micrococcus* spp. The bacteria isolated are of food and public health importance and can be harmful and injurious to the health of the consumer without proper washing and cooking. Håstein *et al.* (2006) and Naiket *et al.* (2015) agree that the occurrence of contaminating bacteria obtained in this study can be attributed to cross-contamination from environment, source, and handling by the sellers. According to the rule of International Association of Microbiology Society, fresh and frozen fish should possess neither *Vibrio* spp. Nor *Salmonella* spp. (Sanjee and Karim, 2016).

Conclusion

The results obtained in this study has revealed that tilapia fishes and crabs sold at Rumuola market are highly contaminated with bacterial count exceeding the recommended limits for seafoods for human consumption and could be potentially hazardous if consumed directly or without proper cooking. *Micrococcus* spp., *Proteus mirabilis*, *Corynebacterium* spp., *Bacillus* spp., *Enterobacter* spp., *Salmonella typhi* and *Citrobacter* spp., isolated in this study are of public health importance, hence proper cooking should be should to avoid cross contamination and eventual food intoxication. Generally, hygienic practices and storage condition are recommended for the marketers.

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