



## **Comparative Bacteriological Evaluation of Frozen and Salt Water Tilapia Fishes (*Oreochromos niloticus* and *Oreochromos aureus*) Sold in Port Harcourt, Rivers State Nigeria**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors DNO and NNO designed the study and wrote the protocol, while author VD performed the statistical analysis, the first draft of the manuscript managed the analyses of the study and literature searches under the strict supervision of author. All authors read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/IJPR/2021/v6i130152

#### Editor(s):

(1) Dr. Jasini A. Musa, University of Maiduguri, Nigeria.

#### Reviewers:

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Complete Peer review History: <http://www.sdiarticle4.com/review-history/62901>

**Original Research Article**

**Received 10 October 2020**  
**Accepted 16 December 2020**  
**Published 16 January 2021**

### **ABSTRACT**

Fishes are highly perishable, and prone to vast variations in quality due to differences in species, feeding habits as well as the environmental and preservation factors. This study Compared the bacteriological quality of Frozen and Salt Water Tilapia Fishes (*Oreochromos niloticus* and *Oreochromos aureus*) sold in Port Harcourt, Nigeria. Total number of sixty (60) samples were evaluated. Frozen tilapia (36) and salt water tilapia fishes (24) were obtained from the three sampling markets using sterile bags which were properly labelled. The samples were transported to the Laboratory for analyses within 2 hours of collection in a thermos box containing ice pack and

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standard microbiological procedures were employed in the bacteriological evaluations. Different parts of the fishes such as Intestine, gills and flesh of the samples were dissected and used for bacteriological analysis. Statistical analyses were carried out using ANOVA and All pairs tukey-kramer. Results obtained from the study showed that the highest number of total heterotrophic bacteria count was obtained from frozen fish gills which was  $7.7 \times 10^6 \pm 0.98$  cfu/g while the least count of  $4.7 \times 10^6 \pm 0.67$  cfu/g was from salt water tilapia fish flesh. Total coliform count ranged from  $3.3 \times 10^4 \pm 0.91$  cfu/g to  $8.0 \times 10^4 \pm 0.44^a$  cfu/g for salt water flesh and frozen fish intestine from different markets respectively. *Listeria* species count ranged from  $1.3 \times 10^4 \pm 0.30$  cfu/g to  $4.3 \times 10^4 \pm 0.57$  cfu/g for salt water fish intestine and frozen fish flesh respectively. Total *Salmonella* count ranged from  $1.0 \times 10^3 \pm 0$  cfu/g to  $6.2 \times 10^3 \pm 1.30$  cfu/g for frozen fish flesh and salt water fish intestine. These values were above the WHO permissible limit. Mean values for all the microbial counts were significantly different at ( $P < 0.05$ ) in the two samples across the sampled markets comparatively, frozen fish has more bacteriological load than salt water fish, this may be attributed to the handling, hygiene storage of the respective fishes as well as storage conditions. *Listeria* species were identified as *L. monocytogenes*, *L. graji*, *L. seeligeri*, *L. ivanovii*, and *L. welshmeri* by genomic studies. While three species of *Salmonella* such as *S. arizonae*, *S. gallinarum*, *S. typhi* were isolated. Other bacterial isolates were identified as *Vibrio spp*, *Bacillus spp* *Staphylococcus spp* *Shigella spp* *Pseudomonas spp*. *Enterobacter spp*. *E. coli*, *Micrococcus spp*. *Acinetobacter spp*. *Klebsiella spp*. This study revealed that fish sold at different markets in Port Harcourt especially frozen fish, is highly contaminated with different kinds of bacterial pathogens which may constitute potential public health hazard due to the unhygienic nature of fish vendors which predisposes frozen fishes to contamination by pathogenic microorganisms. Therefore proper blanching and heating methods should be employed during preparations of fishes to avoid cross contamination and food intoxication/ poisoning before consumption. It is important that all hazard analysis critical control point be adhered to for good production processes.

**Keywords:** Perishable; blanching; hazard analysis; *Listeria* and *Salmonella* species.

## 1. INTRODUCTION

Fish is an important part of a healthy diet due to its high quality protein, and other essential nutrients such as omega 3-fatty acids, low cholesterol level and presence of essential amino acids [1], and its low fat content as compared to other meats [2]. Fish and seafood products constitute an important food commodity in the international trade due to its ever increasing consumption demand. Fish contributes about 60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish [3]. Fish allows for protein improved nutrition because of its high biological value in terms of high protein retention in the body. The contamination often occurs from human and animal sources, and thus, fish and seafood can be involved in the transmission of pathogenic microorganisms and toxins if preparation processes for food are inadequate [4]. Consumption of fish and shellfish may cause diseases due to infection or intoxication; some of these diseases have been specifically associated with pathogens, which are resistant to antibiotics [1]. Microbial contamination on environmental surfaces may be transferred to the food products directly through surface contact or by vectors

such as personnel, pests, air movements or cleaning regimes [5]. Bacteria may also infect the fish from outside during careless handling of fish, its stowing and cutting. Among major external sources of bacterial contamination are ice and salt, crushed ice is known to carry heavy bacterial loads [6]. Generally, fishes are good sources of vitamins B12 and B6. It is also good source of fluorine and iodine which are needed for the development of strong teeth and the prevention of goiter in man [7]. However, availability of these vital nutrients depends to a large extent on the methods of storage such as salting, roasting, drying and freezing. Frozen fish of different types are of great demand by the Nigeria consumers as a relatively cheaper source of animal protein and for the purpose of availability.

Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world [4]. In Nigeria, fish constitute 40% of the animal protein intake [8]. Fish and fish products constitute an important part in the international trade, currently worth more than 50 billion US dollars indicating increasingly consumer interest in commodity.

According to Ogbonna and Inana [9], epidemics of bacterial disease are common in dense populations of cultured food or in aquaculture, thus predisposing the fishes to outbreaks frequently due to poor water quality, organic loading of the aquatic environment, handling and transport of fish, marked temperature changes, hypoxia or other stressful conditions.

Nutritional quality of fish is of major concern to the food processors, consumers, and public health authorities. Provision of safe, wholesome and acceptable fish and fish products, through quality control against contamination is essential for consumers safety. According to Adebayo and Odu [10], fish and shellfish are highly perishable and prone to broad variations in quality due to differences in species, as well as the environmental and feeding habits.

The microbiological flora in the intestines of seafoods such as finfish, shellfish, and cephalopods is quite different being psychotrophic in nature, and to some extent believed to be a reflection of the general contamination in the aquatic environment [11]. According to [12], different fishes are encountered by bacteria as reported for *Pseudomonas angulluseptica* and *Streptococcus* spp. The economic losses due to spoilage are rarely quantified but a report by the US National Research Council Committee (FND/NRC) estimated that one-fourth of the world food supply is lost through microbial activity alone [13].

Fish is one of the most perishable food products, and therefore, quality deterioration of fresh fish occurs rapidly during handling and storage and limits the shelf life of the product [14]. Many pathogenic and spoilage bacteria are able to attach on food contact surfaces, and remain viable even after cleaning and disinfection [15]. The quality of fish could be degraded through a complex process, in which the physical, chemical and microbiological forms of deterioration are involved. The enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the obvious spoilage, and thereby establishes product shelf life [16]. Sensory methods are the most satisfactory for assessing the spoilage and freshness of fish and fishery products [17]. A major goal for the food processing industry is to provide safe, wholesome, and acceptable food to the consumer. [18]. Control of microorganisms

exerted through high level of hygiene and efficient cleaning and disinfection practices during the processing and preservation procedures are essential to meet this goal. The aim of this paper therefore was to determine and compare the bacteriological quality of frozen and salt water tilapia fishes sold in Port Harcourt, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Description of Study Area

Port Harcourt is located in the Niger Delta region; Southern Nigeria. The city is situated between latitudes 3°37' and 3°56' N. and longitude 11°10' and 11°45' E, approximately 50km from the Atlantic coast. Precipitation averages 3,030 mm annual and a temperature average of 23°C. One of Major occupations residents/inhabitants of Port Harcourt is fishing around the marine communities in spite of the various exploitation of oil by multinational companies. Image 1 shows the map of Port Harcourt indicating the sampled markets.

### 2.2 Collection of Sample

A total of Sixty (60) samples of frozen tilapia fish (36), salt water tilapia fish (24) were obtained from three different markets in Port Harcourt city namely; Creek road, Mile one and Rumuokoro markets over a period of six (6) months. Sterile containers were used to store the fishes after purchase and then transported to the Laboratory for analyses within 2 hours of collection in a thermos box containing ice pack.

## 3. BACTERIOLOGICAL ANALYSES OF FISH SAMPLES

### 3.1 Preparation of Samples in the Laboratory

The fish samples were prepared for bacteriological analysis as described by Yagoub [19], The fish samples were rinsed with water to remove surface dirt and then the body surface 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 stomacher lab blender 400 Model No: BA 6021 at 140rev/min for 2minutes as adopted by Omorodion et al. [20].

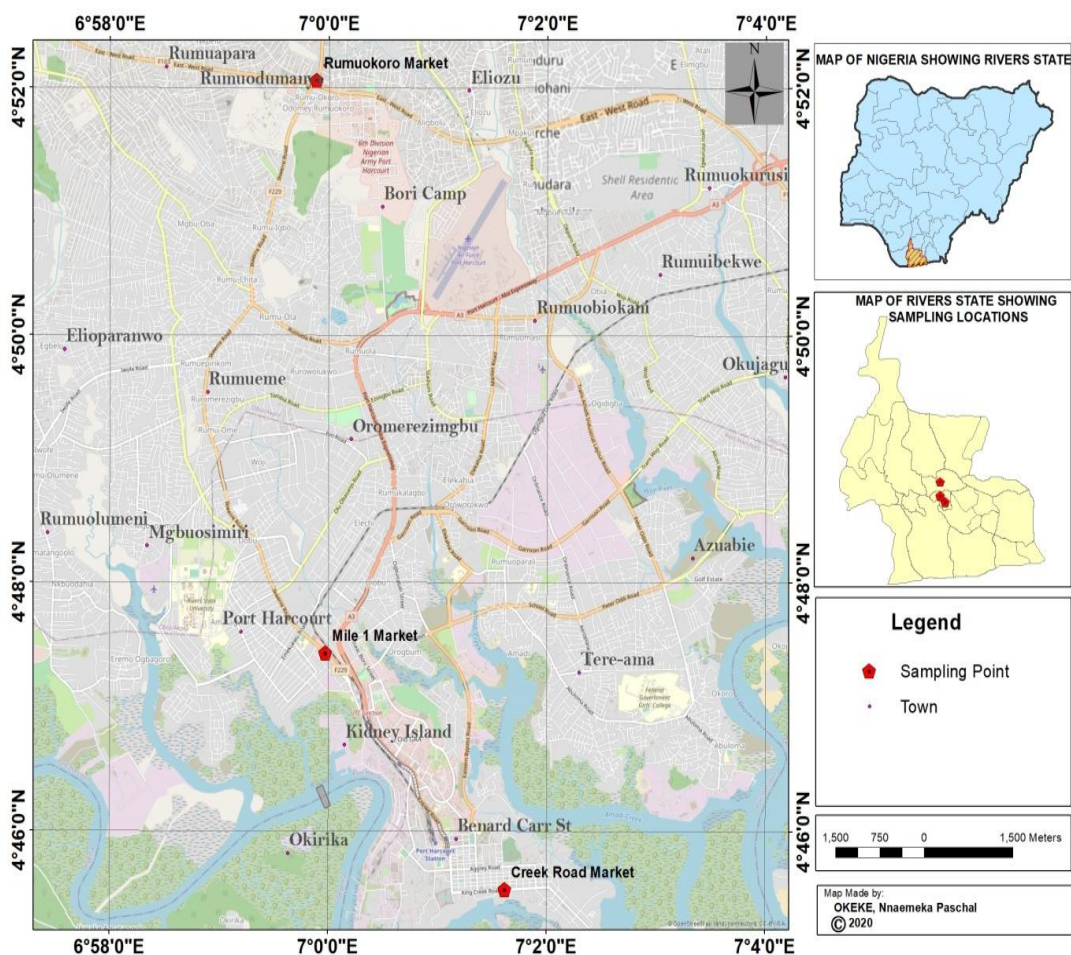


Image 1. Map of Port Harcourt showing the sampled market

### 3.2 Inoculation and Incubation

Aliquots (0.1 ml) of various dilutions were inoculated to surface dried Plates Count Agar (PCA) in duplicates for enumeration of total heterotrophic bacterial population, McConkey agar for total coliform *Salmonella-Shigella* agar (SSA) for *Salmonella* and *Shigella* colonies, and PALCAM selective agar base supplemented with *Listeria* Selective Supplement II (FD063 or FD063I) for *Listeria* population in duplicates and spread evenly with flamed bent glass spreader [1]. The plates were incubated at 37°C for 24 hours.

### 3.3 Enumeration of Isolates on Media

#### 3.3.1 Total heterotrophic bacteria

Total Heterotrophic Bacteria was enumerated as described by Prescott *et al* [21]. Bacterial

colonies that appeared on plate count agar plates (PCA) were counted and the mean expressed as cfu/g. The colony forming unit per gram of the sample was calculated using the formula below;

$$CFU/G = \frac{\text{number of colonies}}{\text{Dilution} \times \text{volume plated}}$$

The discrete colonies were sub cultured on fresh nutrient agar plate in order to isolate pure culture cultures.

#### 3.3.2 Total coliform counts

Total Coliform Counts was enumerated as described by Prescott *et al* [21]. Bacterial colonies that appeared on the MacConkey agar plates were counted and the mean expressed as cfu/g for the samples [22]. The discrete colonies were sub cultured on freshly prepared nutrient agar plate in order to isolate pure cultures.





**Plate 1. Frozen tilapia fish (*Oreochromos niloticus*)**



**Plate 2. Salt water tilapia fish (*Oreochromos aureus*)**

### **3.3.3 *Salmonella* species**

Aliquots (0.1 ml) of serially diluted sample was inoculated in duplicates on surface dried *Salmonella-Shigella* Agar (SSA), for isolation and enumeration of *Salmonella-Shigella*. The inoculums were spread evenly on the plates with flamed bent glass spreader. The plates were incubated at 37°C for 24 hours.

### **3.3.4 *Listeria* species inoculums**

Aliquots (0.1 ml) of serially diluted sample was inoculated in duplicates on surface dried

PALCAM selective agar base supplemented with *Listeria* Selective Supplement II (FD063) Oxoid product, for isolation and enumeration of *Listeria* colonies [1]. They were spread evenly on the plates with flamed bent glass spreader. The plates were incubated at 37°C for 24 hours.

### **3.3.5 Maintenance of pure cultures**

Discrete bacterial colonies that grew on the respective media were sub cultured using streaked plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure cultures. The pure

bacteria cultures were then maintained according to the method adopted by Amadi et al. [24] using ten percent (v/v) glycerol suspension at -4°C.

### 3.3.6 Resuscitation of *Listeria* and *Salmonella* species

For *Listeria* species, resuscitation was done by inoculating ten grams (10) of respective fish parts; flesh, gill and intestine, samples were into 90 ml of Half Fraser broth and incubated at 37°C for 24 h [1]. Ten millilitres (10 ml) of Half Fraser broth containing 24 hour inoculated samples were transferred into full Fraser broth and was incubated at 37°C for 24hour, after which 0.1 ml of Full Fraser was inoculated on PALCAM media and incubated at 37°C for 24hour. For resuscitation of *Salmonella* species, Ten gram (10 g) of the respective samples also were agitated in 90 ml sterile peptone broth and incubated at 37°C for 24 h., for enrichment, then 10 ml of the incubated peptone containing the sample was transferred into Selenite F broth at 37°C for 24 hr for further enrichment while 0.1 ml aliquot was inoculated on *Salmonella-Shigella* Agar (SSA), and incubated at 37°C for 24 hours [1].

### 3.3.7 Identification and characterization of bacterial isolates

Each discrete bacterial colonies that grew on the respective media were characterized based on the cultural morphological, and identified using biochemical test and Analytical Profile Index (API) which is a commercial system to identify different bacteria in which all positive and negative test results are compiled to obtain a profile number, which was then compared using the Bergers's manual of determinative bacteriology for identification of the isolates [23].

### 3.3.8 Statistical analysis

Statistical analyses were carried out using one way ANOVA and All pairs tukey-kramer

## 4. RESULTS AND DISCUSSION

Fish is a good protein source and other elements necessary for the maintenance of healthy body. Exposure to handling, personal hygiene can affect the bacteriological quality. In this study frozen and salt water tilapia fishes were examined for the presence of microorganisms.

Result of bacteriological count shows that frozen fish had the highest number of bacterial count compared to the salt water fish (Tables 1 – 4).

The highest number of bacteria count was obtained from frozen fish intestine while the least count was from fish flesh for both frozen and salt water tilapia fishes across the three markets sampled.

Results of the Mean total heterotrophic bacterial count for frozen tilapia fish ranged from  $5.1 \times 10^6 \pm 0.53$  cfu/g to  $5.9 \times 10^6 \pm 0.99$  cfu/g (flesh),  $6.0 \times 10^6 \pm 0.63$  to  $7.7 \times 10^6 \pm 0.98$  cfu/g (Gill),  $7.1 \times 10^6 \pm 0.68$  to  $7.6 \times 10^6 \pm 0.096$  cfu/g (Intestine), across the markets. Creek road market had the highest bacterial count among the frozen fish analysed in this study with respect to sample locations while the lowest count recorded was from Rumuokoro market. Mean total heterotrophic bacterial count for salt water tilapia fish ranged from  $4.7 \times 10^6 \pm 0.67$  cfu/g to  $5.2 \times 10^6 \pm 0.59$  cfu/g (Flesh),  $5.5 \times 10^6 \pm 1.01$  to  $6.0 \times 10^6 \pm 1.03$  cfu/g (Gill) and  $6.6 \times 10^6 \pm 0.87$  to  $7.0 \times 10^6 \pm 0.83$  cfu/g (Intestine) respectively (Table 1). Creek road market also had highest count among the salt water fishes while Mile 1 market had the least count. Salt water fish was not obtained from Rumukoro market due to unavailability of the fish sample. Fig. 1 shows the comparative mean chart of total heterotrophic bacterial count of frozen fish and salt water fish obtained from the three sampled markets. The result of total coliform showed similar trend for both the bacterial count and source markets (Table 2). The counts obtained in this study were significantly high. These could be attributed to the handling, hygiene and storage conditions in markets. Al-Hindi et al. [25] also found that the total coliform count range in fish were high with increasing values, as the duration of storage increases. From the result of this study, it can be seen that frozen fish sold in the market has high contamination may be as a result of certain factors like temperature which favours some organisms and the character of fish handler, by not maintaining personal hygiene.

Mean *Listeria* spp. count for frozen tilapia fish ranged from  $2.7 \times 10^4 \pm 0.68$  cfu/g to  $2.9 \times 10^4 \pm 0.23$  cfu/g (Intestine),  $3.3 \times 10^4 \pm 0.15$  to  $3.7 \times 10^4 \pm 0.35$  cfu/g (Gill),  $3.8 \times 10^4 \pm 0.44$  to  $4.3 \times 10^4 \pm 0.57$  cfu/g (Flesh ), across the three markets. The highest count was recorded from intestine while the lowest count from the intestine. Comparatively, Creek road market samples had more *Listeria* count than Mile 1 and Rumuokoro markets but there were no significant differences except for the count obtained from the gill that Showed significant difference at  $P < 0.05$ . The highest count recorded from the intestine of frozen fish

part of samples could be attributed to the fact that most *Listeria* spp. are psychrophiles which indicates that they can survive at freezing temperature ranging from - 5°C to - 28°C especially *Listeria monocytogenes*. Handling and exposure to contaminant by vendors could also contribute to the high Listerial load on the flesh part of samples. Mean *Listeria* spp. count for salt water tilapia fish ranged from  $1.3 \times 10^4 \pm 0.22$  cfu/g for both Creek Road and Mile markets, (Intestine),  $1.3 \times 10^4 \pm 0.22$  to  $1.5 \times 10^4 \pm 0.38$  cfu/g (Gill),  $2.5 \times 10^4 \pm 0.45$  cfu/g to  $3.0 \times 10^4 \pm 0.38$  cfu/g (Flesh), across the two markets (Table 3).

Mean total *Salmonella* count for frozen tilapia fish ranged from  $1.0 \times 10^3 \pm 0$  cfu/g to  $1.3 \times 10^4 \times 10^3$  cfu/g (flesh),  $1.0 \times 10^3 \pm 0$  to  $1.6 \times 10^3 \pm 0.58$  cfu/g (Gill),  $1.2 \times 10^3 \pm 0.5$  cfu/g to  $2.0 \pm 1.41$  cfu/g (Intestine), across the three markets. The highest count was recorded from intestine while the lowest count from the flesh. Mean total *Salmonella* count for salt water tilapia fish followed similar trends except that the values were higher compared to that of the frozen fish as shown in (Table 4), high values were higher compared to that of the frozen fish as shown in Table 4. High values of *Salmonella* count could be attributed to the fact that *Salmonella* species are known to be halo tolerant, they maintain a low level of ionic concentrations to synthesize compatible solute to balance the osmotic level inside the cytoplasm with the outer medium. Similar trend were observed across the three markets for the both fishes analysed in this study except for *Listeria* that the highest count was recorded from the flesh part of the sample, though there were significant differences between the bacterial count among the various sample parts analysed in this study but no significant difference at  $P < 0.05$  within samples parts when compared to the various markets [25].

The values of bacteriological counts obtained in this study were above the permissible limit as recommended by regulatory bodies. According to the World Health Organization (WHO) directives on microbial limits, total bacterial count should not exceed  $5 \times 10^5$  colonies per gram of sample. The high bacterial count obtained in this study could be as a result of 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 as early observed by Ogbonna and Inana [5]. The results obtained in this study are in agreement

with the observation of Adebayo et al. [1] who obtained similar bacterial count in their research on microbial quality of different frozen fish sold in Uyo metropolis. Comparatively, frozen fish had more bacteriological load than salt water fish, this may be attributed to the environmental conditions of the respective fishes as well as their proximate composition not only that but also the microbial composition of water has the ability to influence microbial quality of fish that live in water. In this study, Bacteriological count and types of bacteria isolated from salt water fish were significantly different from that of the frozen fish. This may be due to the salinity level of the salt water which inhibits the growth of some organisms that are not salt tolerant [4]. Figs. 2 and 3 shows the percentage occurrence of bacterial isolates in the respective fishes.

The result of morphological, biochemical and API identification shows that five *Listeria* species which include, *L. monocytogenes*, *L. graji*, *L. seeligeri*, *L. ivanovii*, and *L. welshmeri* were isolated and identified in this study. While three species of *Salmonella* such as *S. arizonae*, *S. gallinarum*, *S. typhi* were isolated. Other bacterial isolates were identified as *Vibrio* spp, *Bacillus* spp *Staphylococcus* spp *Shigella* spp *Pseudomonas* spp. *Enterobacter* spp. *E. coli* *Micrococcus* spp. *Acinetobacter* spp. *Klebsiella* spp. the presence of contaminating bacteria obtained in this study could be attributed to cross- contamination from environment, source, and handling by the sellers [12,13]. These results are in line with reports of other studies in Nigeria by various authors [14-17,26,18,2,1]. It showed that the organism isolated from frozen suggest the high level of contamination of water body where these fishes are caught, meaning that water body is not free from microorganisms.

The distribution and percentage occurrence of *Listeria* and *Salmonella* species isolated from the different parts of both frozen and salt water tilapia fishes are presented in Tables 5 to 6 and Figs. 4 and 5 respectively. *Listeria* and *Salmonella* species were resuscitated in this study in order to enhance the growth of all viable species present in the various parts of the samples.

The presence of *Listeria* species in food which colonizes the gastrointestinal tract, especially *Listeria monocytogenes* being the pathogenic species has the unique ability to cause mammalian cells to absorb the organism into their cytoplasm. Using this ability, the pathogen penetrates the intestinal mucosa and can

disseminate by cell-to-cell spread or hematogenously [8]. *L. monocytogenes* has the ability to cross three key barriers; the intestinal barrier, the blood–brain barrier and the fetoplacental barrier, so that it can infect organs such as the brain or uterus, and cause severe life-threatening infections such as meningitis, encephalitis, spontaneous abortion, or miscarriage [19]. Healthy individuals are normally not susceptible to *Listeria monocytogenes*, but it can have severe implications for those with compromised immune systems, such as the elderly, new-borns and pregnant women. According to Cruz et al. [27] *L. monocytogenes* can survive for long periods of time in a seemingly hostile environment such as a food processing facility partially due to its ability to endure various stresses, such as sanitizers, pH and temperature and its ability to form biofilm leading to persistence.

*Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease [21]. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteraemia and enteric fever [22]. The severity of *Salmonella* infections in humans varies depending on the serotype involved and the health status of the human host. Children below the age of 5 years, elderly people and patients with immunosuppression are more susceptible to *Salmonella* infection than healthy individuals. Almost all strains of *Salmonella* are pathogenic as they have the ability to invade, replicate and survive in human host cells, resulting in potentially fatal disease.

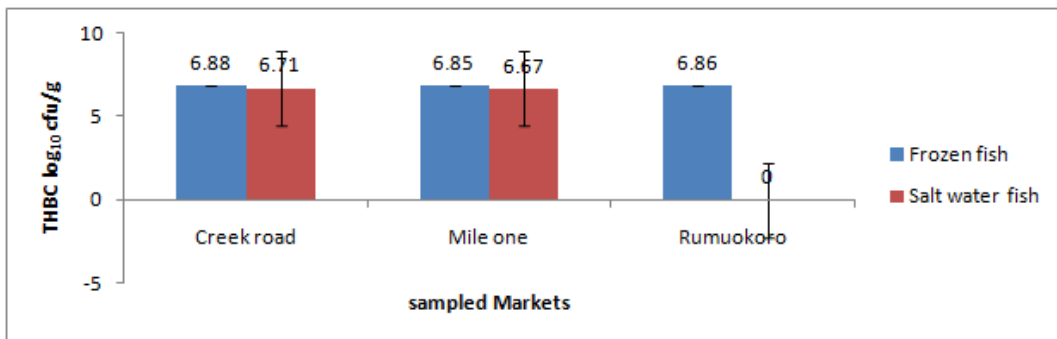


Fig. 1. Comparative mean chart of total heterotrophic bacterial count of frozen fish and salt water fish obtained from the three sampled markets

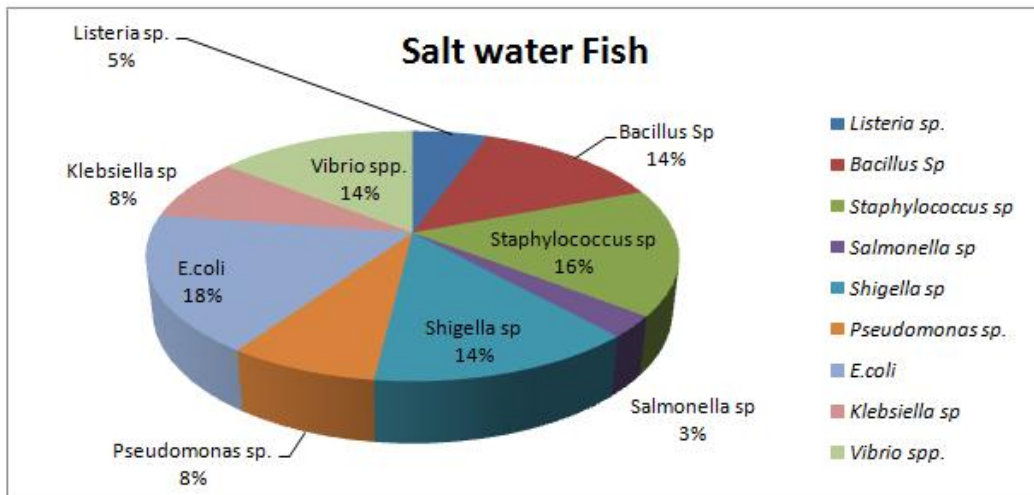


Fig. 2. Distribution and percentage occurrence of bacteria isolated from salt water fish



**Table 1. Total heterotrophic bacterial count of frozen and salt water tilapia fishes from the three sample market**

Sample	Unit	Creek road	Mile one	Rumuokoro	P value	Significant Difference
Frozen Fish Flesh	cfu/g	5.9x10 <sup>6</sup> ±0.99 <sup>ab</sup>	5.7 x10 <sup>6</sup> ±1.06 <sup>abcd</sup>	5.1 x10 <sup>6</sup> ±0.53 <sup>abc</sup>	0.364	No
Frozen Fish Gill	cfu/g	7.5x10 <sup>6</sup> ±0.8 <sup>ab(x)</sup>	7.7 x10 <sup>6</sup> ±0.98 <sup>ab(x)</sup>	6.0 x10 <sup>6</sup> ±0.63 <sup>ab(y)</sup>	0.014	Yes
Frozen Fish Intestine	cfu/g	7.6x10 <sup>6</sup> ±0.96 <sup>ab</sup>	7.1 x10 <sup>6</sup> ±0.68 <sup>abc</sup>	7.3 x10 <sup>6</sup> ±0.84 <sup>a</sup>	0.555	No
SW Fish Gill	cfu/g	6.0 x10 <sup>6</sup> ±1.03 <sup>ab</sup>	5.5 x10 <sup>6</sup> ±1.01 <sup>bcd</sup>	ND	0.463	No
SW Fish Intestine	cfu/g	7.0 x10 <sup>6</sup> ±0.83 <sup>ab</sup>	6.6 x10 <sup>6</sup> ±0.87 <sup>abcd</sup>	ND	0.440	No
SW Fish Flesh	cfu/g	5.2 x10 <sup>6</sup> ±0.59 <sup>b</sup>	4.7 x10 <sup>6</sup> ±0.67 <sup>cd</sup>	ND	0.215	No
P value		0.0006*	<0.0001*	0.0003*		
Significant Difference		Yes	Yes	Yes		

Means with different superscript (<sup>abcde</sup>) shows Significant Difference along columns; Means with different superscript (<sup>xyz</sup>) Significant Difference across rows; ND =Not determine

**Table 2. Total Coliform count of frozen and salt water tilapia fishes from the three sample market**

Sample	Unit	Creek road	Mile one	Rumuokoro	P value	Significant Difference
Frozen Fish Flesh	cfu/g	5.4x10 <sup>4</sup> ±0.69 <sup>bcd(x)</sup>	4.5 x10 <sup>4</sup> ±0.39 <sup>bc(xy)</sup>	4.2 x10 <sup>4</sup> ±0.42 <sup>bc(y)</sup>	0.011*	Yes
Frozen Fish Gill	cfu/g	6.6 x10 <sup>4</sup> ±0.58 <sup>ab(x)</sup>	5.7 x10 <sup>4</sup> ±0.55 <sup>ab(y)</sup>	5.2 x10 <sup>4</sup> ±0.27 <sup>ab(y)</sup>	0.002*	Yes
Frozen Fish Intestine	cfu/g	8.0 x10 <sup>4</sup> ±0.44 <sup>a(x)</sup>	6.4 x10 <sup>4</sup> ±0.65 <sup>a(y)</sup>	6.1 x10 <sup>4</sup> ±0.44 <sup>a(y)</sup>	0.002*	Yes
SW Fish Gill	cfu/g	5.0 x10 <sup>4</sup> ±0.61 <sup>cd</sup>	4.3 x10 <sup>4</sup> ±0.29 <sup>bcd</sup>	ND	0.041*	Yes
SW Fish Intestine	cfu/g	5.9 x10 <sup>4</sup> ±0.43 <sup>bc</sup>	4.7 x10 <sup>4</sup> ±0.51 <sup>bc</sup>	ND	0.004*	Yes
SW Fish Flesh	cfu/g	4.1 x10 <sup>4</sup> ±0.81 <sup>de</sup>	3.3 x10 <sup>4</sup> ±0.91 <sup>cd</sup>	ND	0.211	No
P value		<0.0001	<0.0001	<0.0001		
Significant Difference		Yes	Yes	Yes		

Means with different superscript (<sup>abcde</sup>) shows Significant Difference along columns; Means with different superscript (<sup>xyz</sup>) Significant Difference across rows; ND =Not determine

**Table 3. *Listeria* count of the various sampled parts from the three sampled markets**

Sample	Unit	Creek road	Mile one	Rumuokoro	P value	Significant Difference
Frozen Fish Flesh	cfu/g	2.9 x10 <sup>4</sup> ±0.7 <sup>b</sup>	2.7 x10 <sup>4</sup> ±0.68 <sup>b</sup>	2.9 x10 <sup>4</sup> ±0.23 <sup>b</sup>	0.758	No
Frozen Fish Gill	cfu/g	3.7x10 <sup>4</sup> ±0.35 <sup>ab(x)</sup>	3.3 x10 <sup>4</sup> ±0.15 <sup>ab(y)</sup>	3.3 x10 <sup>4</sup> ±0.27 <sup>ab(y)</sup>	0.045*	Yes
Frozen Fish Intestine	cfu/g	4.3 x10 <sup>4</sup> ±0.57 <sup>a</sup>	3.8 x10 <sup>4</sup> ±0.44 <sup>a</sup>	3.9 x10 <sup>4</sup> ±0.34 <sup>a</sup>	0.193	No
SW Fish Gill	cfu/g	1.5 x10 <sup>4</sup> ±0.38 <sup>c</sup>	1.3 x10 <sup>4</sup> ±0.30 <sup>cd</sup>	ND	0.435	No
SW Fish Intestine	cfu/g	1.3 x10 <sup>4</sup> ±0.21 <sup>c</sup>	1.3 x10 <sup>4</sup> ±0.22 <sup>cd</sup>	ND	0.773	No
SW Fish Flesh	cfu/g	3.0 x10 <sup>4</sup> ±0.38 <sup>b</sup>	2.5 x10 <sup>4</sup> ±0.45 <sup>b</sup>	ND	0.107	No
P value		<0.0001	<0.0001	<0.0001		
Significant Difference		Yes	Yes	Yes		

Means with different superscript (<sup>abcde</sup>) shows Significant Difference along columns; Means with different superscript (<sup>xyz</sup>) Significant Difference across rows; ND =Not determine

**Table 4. *Salmonella* Count of the various sampled parts from the three sampled markets**

Sample	Unit	Creek road	Mile one	Rumuokoro	P value	Significant Difference
Frozen Fish Flesh	cfu/g	1.0 x10 <sup>3</sup> ±0 <sup>b</sup>	1.3x10 <sup>4</sup> x10 <sup>3</sup> ±0.58 <sup>b</sup>	1.0 x10 <sup>3</sup> ±0	0.833	No
Frozen Fish Gill	cfu/g	1.6x10 <sup>3</sup> ± 0.58 <sup>b</sup>	1.0 x10 <sup>3</sup> ±0 <sup>b</sup>	1.0 x10 <sup>3</sup> ±0	0.218	No
Frozen Fish Intestine	cfu/g	2.0±1.41 <sup>b</sup>	1.5 x10 <sup>3</sup> ±1.0 <sup>b</sup>	1.2 x10 <sup>3</sup> ±0.5	0.586	No
SW Fish Intestine	cfu/g	6.2x10 <sup>3</sup> ±1.30 <sup>a</sup>	4.0x10 <sup>3</sup> ±0.71 <sup>a</sup>	ND	0.011*	Yes
SW Fish Flesh	cfu/g	1.5 x10 <sup>3</sup> ±0.58 <sup>b</sup>	1.5x10 <sup>3</sup> ±0.70 <sup>b</sup>	ND	1.0	No
SW Fish Gill	cfu/g	3.6 x10 <sup>3</sup> ±1.14 <sup>b</sup>	1.6x10 <sup>3</sup> ±0.55 <sup>b</sup>	ND	0.008*	Yes
P value		<0.0001*	0.0006*	0.646		
Significant Difference		Yes	Yes	No		

Means with different superscript (<sup>abcde</sup>) shows Significant Difference along columns; Means with different superscript (<sup>xyz</sup>) Significant Difference across rows; ND =Not determine

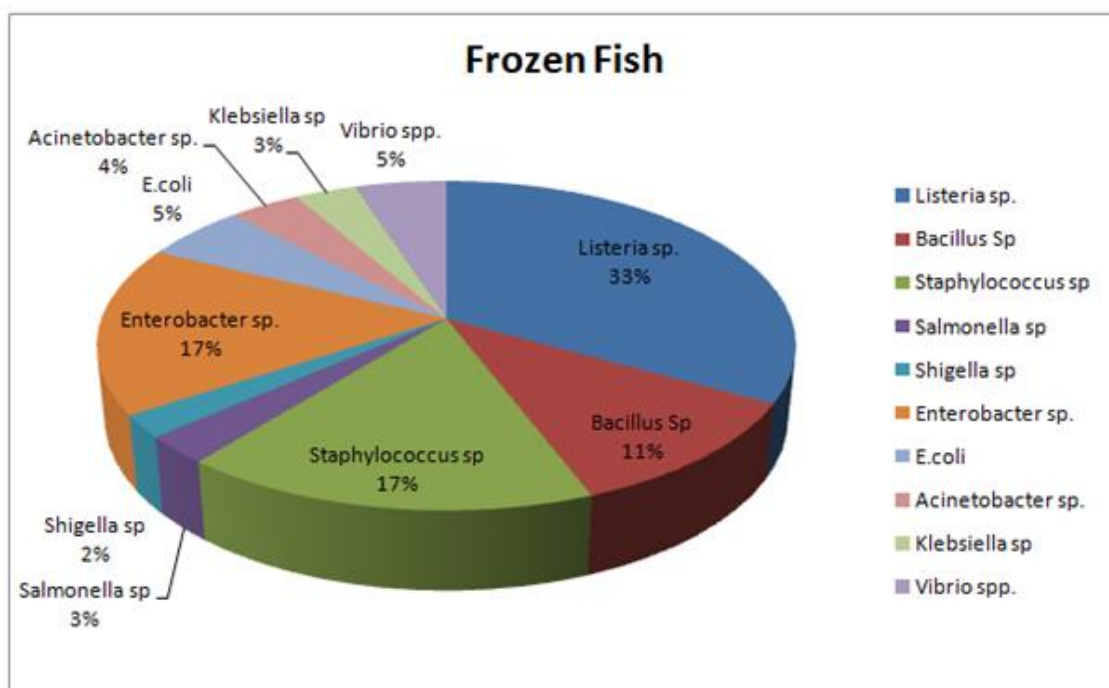


Fig. 3. Distribution and percentage occurrence of bacteria isolated from frozen fish

Table 5. Distribution and percentage occurrence of *Listeria* in the various parts of frozen and salt water fishes from the three sampled markets after Resuscitation

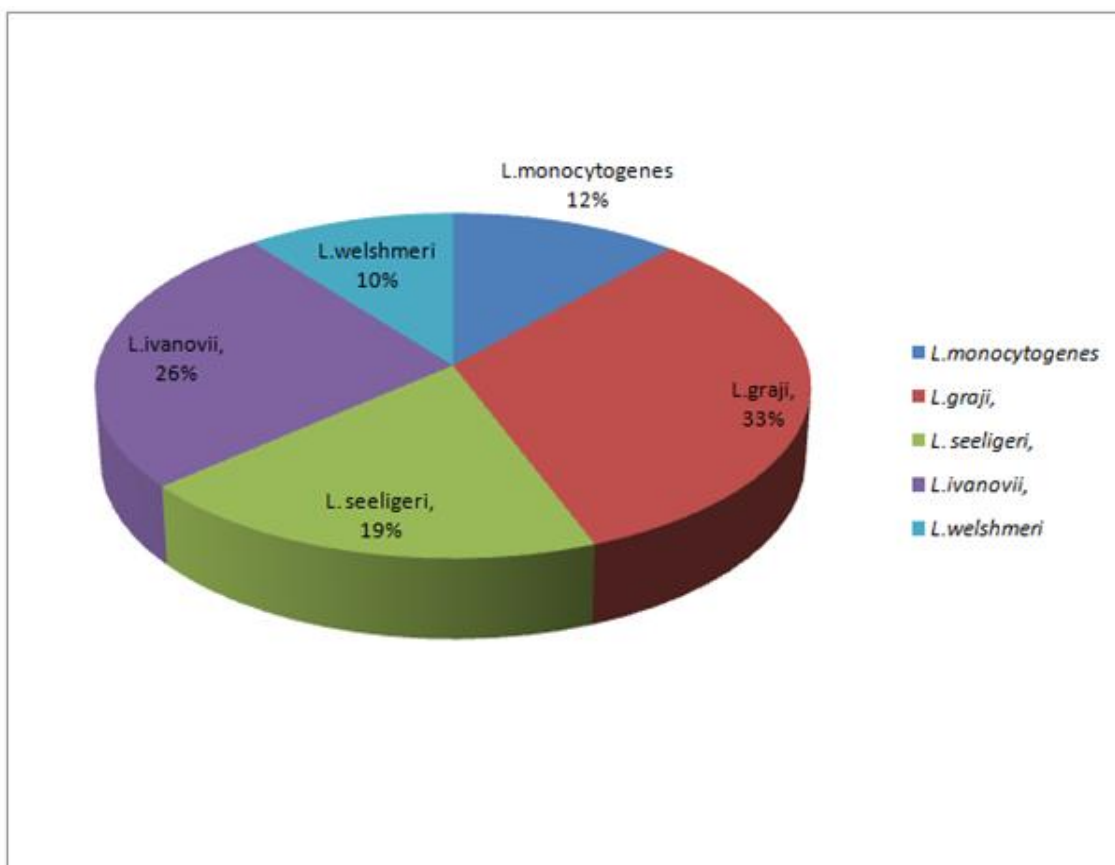
Isolates	Frozen tilapia fish			Salt water tilapia fish			T F	%F
	Flesh	Gill	Intestine	Flesh	Gill	Intestine		
<i>L. monocytogenes</i>	+ (12)	+(10)	+(8)	+(2)	+(2)	- (0)	34	11.56
<i>L. graji,</i>	+ (28)	+(20)	+(16)	+ (15)	+(10)	+(7)	96	32.65
<i>L. seeligeri,</i>	+ (19)	+(15)	+(7)	+(8)	+(5)	+(3)	57	19.39
<i>L. ivanovii,</i>	+ (19)	+(26)	+(17)	+(6)	+(5)	+(3)	76	25.85
<i>L. welshmeri</i>	- (0)	+(5)	+(6)	+(9)	+(6)	+(5)	31	10.54
Total	78	76	54	40	28	18	294	

Table 6. Distribution and percentage occurrence of *Listeria* in the various parts of frozen and salt water fishes from the three sampled markets after resuscitation

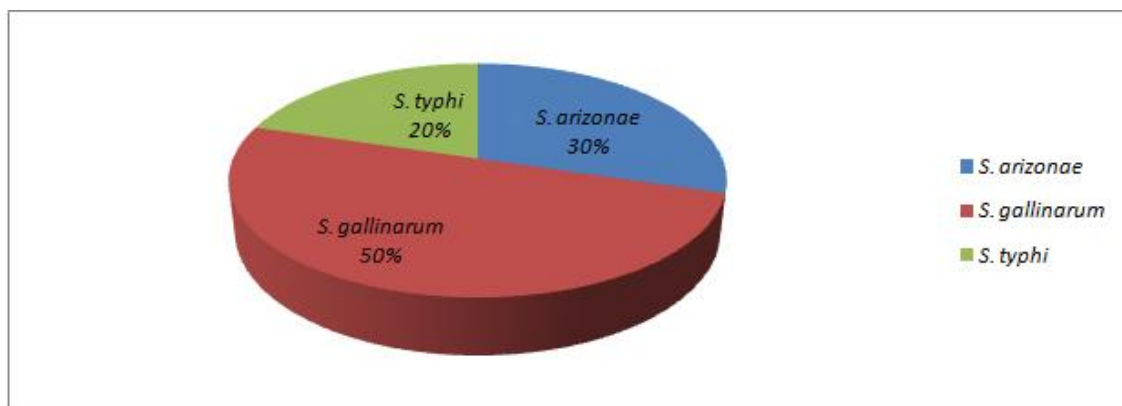
Isolates	Frozen tilapia fish			Salt water tilapia fish			T F	%F
	Flesh	Gill	Intestine	Flesh	Gill	Intestine		
<i>S. arizonae,</i>	- (0)	+ (4)	+ (5)	+ (2)	+ (3)	+ (5)	19	30
<i>S. gallinarum</i>	+ (3)	+ (4)	+ (5)	+ (5)	+ (6)	+ (9)	32	50
<i>S. typhi</i>	- (0)	+ (2)	+ (2)	+ (1)	+ (3)	+ (5)	13	20
Total	3	10	12	8	12	19	64	

Apart from the *Listeria*, *Salmonella* and other enteric organisms which serve as indicator of pathogens, *Staphylococcus* species isolated in this study are known enteritoxic producing

agent and a microorganism which is poisonous. This is in agreement with the previous reports of some other workers [1,15]. Similar bacteria isolated in this study were also reported for fish



**Fig. 4. Percentage occurrence of *Listeria* spp. isolated from frozen and salt water tilapia fishes after resuscitation**



**Fig. 5. Percentage occurrence of *Salmonella* spp. isolated from frozen and salt water tilapia fishes after resuscitation**

and fish products by other researchers [23,24] and [4]. *Escherichia coli* is the most common contaminant and is often encountered in high numbers. *Salmonella* species especially *S. typhi*

causes Salmonellosis which is reported as one of the main cause of diarrhoeal diseases globally [25]. The disease is also associated with enteric fever, including typhoid which is a potentially fatal

systemic illness bedevilling many developing countries.

The disease is estimated to affect nearly 17 million people with over 150,000 deaths occurring annually. Salmonellosis is also beginning to emerge as a food borne infection characterized by significant economic and public health hazard with global ramifications [25]. High prevalence of the disease is directly related to poor sanitation and hygiene,

## 5. CONCLUSION AND RECOMMENDATIONS

The result obtained in this study are very essential to public health, This study revealed that both frozen fish and salt water fishes sold at different markets in Port Harcourt are highly contaminated with different kinds of bacterial pathogens such as *Listeria monocytogenes*, *Salmonella* species, *Vibrio* spp, *Bacillus* spp *Staphylococcus* spp *Shigella* spp *Pseudomonas* spp. *Enterobacter* spp. *E. coli* *Micrococcus* spp. *Acinetobacter* spp. and *Klebsiella* spp. which may constitute potential public health hazard due to the unhygienic nature of fish Vendors which predisposes frozen fishes to contamination by pathogenic microorganisms. Comparatively, frozen tilapia fish harbour more microbial load than the salt water tilapia fish. Therefore proper blanching and heating methods should be employed during preparations especially the frozen fishes to avoid cross contamination and food intoxication/ poisoning before consumption. It is important that all hazard analysis critical control point be adhered to for good production processes.

## ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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