



INCIDENCE OF PATHOGENIC BACTERIA IN FROZEN MACKEREL (*Scomber scombrus*) RETAILED IN OTA METROPOLIS

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ABSTRACT

The present study aimed to evaluate the hygienic quality and freshness of fish Mackerel “*Scomber scombrus*” through the investigation of the occurrence of bacteria which is an indicator for fish quality. Aquaculture products (fish and fish products) can harbour pathogenic bacteria, which are part of the natural micro-flora of the environment. Frozen mackerel fish (*S. scombrus*) retailed in Ota, Ogun state, Nigeria had no documented research report till date. Fish samples were randomly selected from three (3) different depot in Sango, Ota. From this, ten (10 g) samples were used for microbiological analysis. The bacterial loads of the samples were determined using the spread plate technique. Isolates were morphologically and biochemically identified and characterized according to the identification scheme of Bergey’s Manual of Determinative Bacteriology. *In-vitro* assay showed three predominant bacterial contaminants as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* spp. occurring at 75, 14 and 11% respectively. The mean bacterial load of the isolates from this study was found to be markedly higher ($1.252 \pm 1.03 \times 10^6$ CFU g⁻¹) than the recommended official public health and safety standard value between 5.0×10^5 and 1.0×10^6 CFU g⁻¹. Consequently, microorganisms could contaminate fish and therefore a source of food poisoning. Harvesting, handling and cooking of frozen fish especially mackerel (*S. scombrus*) should be done properly so as to reduce the bacterial load. Hence, eating of frozen *S. scombrus* raw or half-boiled should be totally discouraged to eliminate zoonotic infections from fish especially in this environment.

Keywords: *Scomber scombrus*, pathogenic bacteria, bacterial load enumeration, identification

INTRODUCTION

Among the food resources of the world, fish and fishery products are very important as sources of protein especially in some countries that are unsuitable for livestock production and it is estimated that more than 30 % of fish for human consumption comes from aquaculture (Karthiga Rani *et al.*, 2016). Fish is one of

the main food components of humans for centuries and presently occupying an important part of the diet of many countries. Protein plays an important role in the life of man, the family and the nation (Eze *et al.*, 2011).

Fish constitute the cheapest source of animal protein in Africa. It is one of the main food components of humans for many centuries and still constitutes an important part

of the diet of many countries. The advantage of fish as a food resulted from its easy digestibility and high nutritional value (Eze *et al.*, 2011). Since 70% of the earth's surface is covered by water, there are plenty sources to harvest fish from. Fishes are found in different waters. Some are found in fresh water while some are found in salt water (sea and oceans) (Eze *et al.*, 2011). However, the type of microorganisms found associated with a particular fish depends on the water it was found (Eze *et al.*, 2011). It is noteworthy that pollution can also significantly affect the shelf life of the marine fish because the fish species inhabiting polluted waters have already compromised their original health status (Adeyemo, 2003). Generally, in the first couple of hours after death, changes in fish are mainly due to biochemical processes, however after several hours, few bacteria penetrate the flesh where they degrade tissue components producing the unpleasant odours and flavours that are associated with spoilage (Okoro *et al.*, 2010). At room temperature, seafood is likely to deteriorate very fast. Freezing and storage at low temperatures slows down bacterial growth and deterioration of fish through some enzymatic and chemical changes that progress slowly (Okoro *et al.*, 2010). Time and temperature are the most critical factors to control the microbial quality of food. Time and temperature is used to ensure that seafood retains high freshness quality as long as possible (Adams and Moses, 2008).

Bacteria pathogens associated with fish are classified into two (Kvenberg, 1991; Eze *et al.*, 2011): non-indigenous and indigenous bacteria pathogens. The non-indigenous pathogen contaminates fish or fish's habitat in one way or the other, it can either be by sewage waters and this would result in the increase of enteric pathogen such as *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* species, *Shigella* species, and *Escherichia coli* etc. The indigenous bacteria pathogens are those naturally living in the fish habitat, since organisms are

found ever where (ubiquitous), there are microorganisms present and associate with the fishes either by obtaining nutrients from the fishes and not causing harm to the fishes (commensalism) or they are obtaining nutrients and harm the fish (parasites). These organisms that naturally associate with fish may be pathogenic to human health, examples of these organisms include the *Vibrio* species, *Aeromonas* species etc. Eze *et al.* (2011) also listed some organisms likely to cause food-borne diseases when present in ready-to-eat sea foods. The list included *Staphylococcus aureus*, *Salmonella*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Shigella*, *Aeromonas*, *Yersenia*, and *Pseudomonas*.

Fishes have high water content and freeze between temperatures of 0 and 3°C with an average of about 2°C. Freezing kills some bacteria, but the ones not killed will grow upon thawing (Eze *et al.*, 2011). Such bacteria like *Pseudomonas* species, *Moraxella* species, *Alcaligenes* species, *Flavobacterium* species, etc can survive freezing temperature and will resume growth when thawed (Eze *et al.*, 2011). At a temperature of 3°C or above, species of *Clostridium botulinum* can survive freezing and may grow and produce toxins (Eze *et al.*, 2011). FAO (1989) report showed that fishes become contaminated at sea prior to freezing due to difficulty in designing the plant that would be able to operate satisfactorily at all time in adverse weather conditions at sea. The report further indicated that the method of catching fish contributes to the bacterial load of frozen fish and observed that trawling of fish net along the bottom sediments of water for a long time could result in exposing the fish to a high bacterial contamination.

In view of the various ways fishes could be contaminated with microorganisms, the present research was therefore aimed at finding out if frozen fish consumed in this locality is contaminated with bacteria pathogens, and if so, to identify the major bacteria contaminants.

Hence the specific objectives of this study therefore were to ascertain and identify the type of pathogenic bacteria associated with frozen fish sample sold in fish depot in Sango-Ota metropolis and assess the incidence of these bacterial pathogens in the fish sample sold at frozen fish depots.

MATERIALS AND METHOD

Collection of samples

Frozen mackerel fish samples, were randomly purchased in sterile polythene bags from three selected depots in Sango, Ota and conveyed to Bells University Microbiology laboratory for analysis. Adopting the method described by Eze *et al.* (2011), each of the frozen fish samples was aseptically chunked into bits and homogenized.

Sample preparation was made, about 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples were crushed into small pieces in a sterile mortar with about 10 ml sterile distilled water. From the crushed sample, 1 ml aliquot was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of sterile distilled water giving a 1:10 dilution.

ESTIMATION OF BACTERIA LOAD

The bacteria load was estimated using the method described by Collins *et al.* (1989) for microbial count.

Nine (9) millilitres of sterile water was poured aseptically into five sterile tubes each, and 1 ml of the original crushed fish sample (stock) was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. This procedure continued until the fifth test tube. The stock was therefore diluted from 10^{-1} to 10^{-5} for each fish sample.

The nutrient agar were inoculated in duplicates with 0.1

ml of the diluted solution (10^{-2} to 10^{-5}) using glass spreader technique. All plates were incubated at 37°C for 24 h before colony enumeration and isolation. The temperature was chosen to differentiate the mesophiles which constitute most medically important pathogenic bacteria (Eze *et al.*, 2011).

Enumeration, Isolation and Identification of bacterial isolates

The total bacteria count of frozen fish samples were determined first by inoculating serial dilution from 10^{-2} to 10^{-5} into nutrient agar which is a general purpose medium for culturing less fastidious bacteria. The plates then were counted using the colony counter. The mean colony counts on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (CFU g⁻¹). Colonies were then isolated based on their cultural properties such as pigmentation, shape, and elevation, smooth and rough margin. Discrete suspected colonies

Calculation of mean colony forming unit per gram (CFU g⁻¹)

The mean colony forming unit per gram (CFU g⁻¹) denoted by ($\bar{X}$) was calculated as $\Sigma fx / \Sigma f$, where Σfx is the sum of the products of number of colonies and the colony forming unit per gram (f and X); while Σf is the summation of the number of colonies. were further sub-cultured and Gram's staining was carried out to characterize their morphology. Identification of isolates was carried out based on the method described by Eze *et al.* (2011)

RESULTS AND DISCUSSION

The total viable bacterial count of the frozen mackerel fish samples expressed in colony forming unit per gram (CFU g⁻¹). The nine (9) crushed frozen fish samples designated S1 to S9 gave visible clear colonies in plates with 10^{-4} dilution factor. Additionally, the mean number of colony for this factor ranged between 4 and 18, whereas the highest and lowest population in the colony

forming unit per gram for this study were $1.8 \pm 1.4 \times 10^6$ in fish sample S5 and $4.0 \pm 2.8 \times 10^5$ in fish sample S9 respectively (Table 1)

be motile but it does not have the ability to ferment glucose, oxidase and catalase are positive and coagulase negative.

Table 1: Mean Bacterial colony counts $\pm$ SD (CFUg⁻¹) of the frozen fish samples of the selected cold stores

Fish sample	Dilution Factor	Average number of Colonies (f)	Colony forming unit of g (X)	fX
S1	10 ⁴	16	$1.6 \pm 4.2 \times 10^6$	2.56×10^7
S2	10 ⁴	12	$1.2 \pm 2.8 \times 10^6$	1.44×10^7
S3	10 ⁴	8	$8 \pm 1.4 \times 10^5$	6.4×10^6
S4	10 ⁴	4	$4 \pm 1.4 \times 10^5$	1.6×10^6
S5	10 ⁴	18	$1.8 \pm 1.4 \times 10^6$	3.24×10^7
S6	10 ⁴	14	$1.4 \pm 2.8 \times 10^6$	1.96×10^7
S7	10 ⁴	10	$1 \pm 1.4 \times 10^6$	1×10^7
S8	10 ⁴	6	$6 \pm 1.4 \times 10^5$	3.6×10^6
S9	10 ⁴	4	$4 \pm 2.8 \times 10^5$	1.6×10^6
		$\Sigma f = 92$		$\Sigma fX = 1.152 \times 10^8$

$$(\text{Mean}) \bar{X} = \Sigma fx / \Sigma f = 1.152 \times 10^8 / 92 = 1.252 \pm 1.03 \times 10^6 \text{ cfu g}^{-1}$$

Cultural, morphological and biochemical characteristics of bacterial isolates

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas* spp. were the predominant pathogenic bacteria species found associated with the fish sample (Table 2). The *S. aureus* isolated was also found to be positive for coagulase, catalase, and sugar fermentation tests.

The strains were also mannitol-positive but found to be indole and motility negative gram-positive cocci bacteria. The result also revealed that *E. coli* strains sampled displayed different strains with differential results with respect to sucrose fermentation. Generally, they were coagulase negative, and catalase positive and fermented lactose, glucose or fructose. They also exhibited positive reactions to mannitol, indole and motility. They reacted negatively to gram-strains and were therefore characterized as bacilli bacteria species. *Pseudomonas species* were found to be gram negative bacilli, it does not produce Hydrogen sulphide, it cannot breakdown tryptophan to indole, it is found to

Frequency of occurrence of the various isolates

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas* spp., were the common pathogenic bacteria found associated with frozen fish mackerel in Ota. *S. aureus* was isolated from four (4) out of twenty-eight (28) inoculated plates. This represented 14% of the isolated samples. *E. coli* was isolated from twenty-one (21) of the inoculated plates and therefore represent 75% of the isolated microorganisms. The least was *Pseudomonas* spp. which represented 11% of the isolated organism having been isolated from three (3) of the inoculated plates (Table 3). The presence of *S. aureus* was attributed to the contamination of the fish samples by man's activity. Clauca and Ward (1996); Sichewo et al. (2014) recorded that *S. aureus* seldom, if ever, occurred as natural micro-flora of fish and shellfish; its main habitat is humans and animals and is found mostly in the nose, throat and skin of healthy individuals.

This suggests that frozen fish with this pathogen post-harvest must have been contaminated through handling.

Contamination of frozen fish with *E. coli* also suggested that one or more of enteric pathogens have gained access to the fish.

Table 2: Morphological and biochemical characteristics of the bacterial isolates

Gram reaction	Citrate	Catalase	Oxidase	Coagulase	Indole	Lactose	Sucrose	Glucose	Motility	Probable Microorganisms
Positive cocci	+	+	–	+	–	+	+	+	–	<i>Staphylococcus aureus</i>
Negative bacilli	–	+	–	–	+	+	+	+	+	<i>Escherichia coli</i>
Negative bacilli	+	+	+	–	–	–	–	–	+	<i>Pseudomonas</i> spp.

Key: +, Positive; – , Negative

This organism (*E. coli*) is particularly useful as the reliable indicator of faecal contamination when appeared in small numbers or as an indicator of mishandling when appeared in large numbers (Silliker and Gabis, 1976; Chao *et al.*, 2003; Eze *et al.*, 2011). Besides, the co-existence of *E. coli* and the gram-positive. *S. aureus* which cause food poisoning could have been transferred through contact with skin of handlers. Clauca and Ward (1996) further suggested pathogens when present in food like *Staphylococcus aureus*, *Salmonella*, *Shigella*, and *Pseudomonas* are most likely to cause food-borne diseases. The presence of diverse enteric bacteria in fish indicates its

1.03 x 10⁶ CFU g⁻¹) (Table 1) than the recommended official public health and safety standard value of between 5.0 x 10⁵ and 1.0 x 10⁶ CFU g⁻¹, approved by The National Agency for Food and Drug Administration and Control (NAFDAC). This raises concern about the hygienic status of the production and point of sale environment, also the microbiological standard set by NAFDAC. Although cannot be taken as an absolute figure. This is because the number and type of bacteria found on frozen fish is dependent on many factors, of which, source of the fish contributes the major factor and supports. On the contrary, Gilbert *et al.* (1996); Karthiga

Table 3: The prevalence of isolated bacteria in the culture samples

Isolates	Number of colonies	Occurrence of the colonies (%)
<i>Staphylococcus aureus</i>	4	14
<i>Escherichia coli</i>	21	75
<i>Pseudomonas</i> spp.	3	11
Total	28	100

strongly recommended. A high population of bacteria in food indicates the general quality of the food and the degree of spoilage it might have undergone. The mean viable count of the organisms from the study was found to be higher (1.252 ±

temperature during storage, severity of freezing process with respect to lethality to microorganisms and contamination by handlers and market sellers. Brooks *et al.* (2004) however, concluded that one of the sources of

infection is contaminated food. It is therefore recommended that proper processing of frozen fish samples be carried out before consumption. Although only a few infectious agents in fish are able to infect humans, some exceptions such as *Salmonella* exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub, 2009).

CONCLUSION

The determination of bacterial contamination of frozen mackerel (*Scomber scombrus*) fish carried out in this study was necessary in safeguarding public health. This is because frozen fish which is contaminated with faecal material before or during harvest, may cause outbreak of intestinal infectious disease such as typhoid fever. The bacteria isolation assay result revealed the presence of commensal bacterial of intestinal origin, viz: *E. coli*.

The *S. aureus* however is of external sources. The microbial population was found to be higher than the approved safety standard by NAFDAC. Since these microorganisms could contaminate fish and therefore a source of food poisoning; harvesting, handling and cooking of frozen fish especially mackerel (*S. scombrus*) should be done properly so as to reduce the bacterial load. In this case, eating of frozen *S. scombrus* raw or half-boiled should be discouraged to eliminate zoonotic infections from fish especially in this area. This study therefore is intended to provide basic information about these microorganisms likely to cause food-borne disease when present in frozen fish that is ready-to-eat.

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