

Bacteria Floral Isolation in Retailed Frozen Mackerel (*Scomber scombrus*) collected from Akure Metropolis

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Abstract

Distribution chains of fish can subject fish to environmental influences such as bacterial flora. Hence, fish examination was conducted on highly desired frozen *Scomber scombrus* fish samples collected from three different market stalks in Akure South Local Government, Ondo State, Nigeria to ascertain and identify bacterial contaminants of frozen fish. Bacterial load of fish samples was determined on Eosine Methylene blue agar, Mannitol salt agar, Salmonella Shigella agar using streak plate method. Identification and characterization of various isolates were based on gram-staining technique and biochemical tests on *Scomber scombrus* fish collected. In-vitro assay result revealed that samples were contaminated by six bacteria species, which include *Staphylococcus aureus* (20.98%), *Bacillus subtilis* (12.90%), *Staphylococcus epidermis* (20.98%), *Salmonella typhi* (10.49%), *Escherichia coli* (19.10%) and *Shigella dysenteriae* (5.55%).

Keyword:

Bacteria flora, Frozen fish, Scomber scombrus, Environmental Influence, Agar

Introduction

Fish and fish products constitute more than 60% of the total protein intake in adults in Nigeria, especially in the rural areas, and they supply a good balance of protein, vitamins and minerals. Fish has a caloric content which enhances its value, as well as its role in the nation's nutrition (Akinneye, *et al.*, 2009).

Marketing of fish in Nigeria is mostly carried out by fishmongers at ambient temperature (Okoro, *et al.*, 2010), a condition that favours contamination by and proliferation of microorganisms. Thus, consumption of fish may cause diseases due to infection (Okoro, *et al.*, 2010; FDA 2011). Fish contamination should be prevented to ensure its safety for consumption. Many bacteria that are potential spoilers abound in the surface slime, gill and intestine of live fish but the natural defences prevent invasion while the fish is alive. Multiplication and invasion occurs soon after death of fish (Agbolagba and Uwagbai, 2011).

The microbiological contamination concern had shown high loads of unspecified spoilage bacteria like *Salmonella sp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Gram and Bon, 2010). Initial micro-flora on fish is directly related to the surrounding aquatic environment, while the bacterial flora in the gastrointestinal tract corresponds to the condition of the fish (Liston, 2009). It has been well known that fish can harbour human pathogenic bacteria particularly coli form group (Gram and Bon, (2010). Pathogens may be found on raw fish as a result of poor sanitary practices on the harvest vessel, unclean hand, contaminated water, or sewage (FDA, 2011).

Materials and Methods

Selection of Sample Site

Oja – Oba, Isinkan and Stateline area stalls were the three markets environment selected for the study within Akure South Local Government, Ondo State, Nigeria. Collection of samples were limited to three respective frozen fish retailers per market area, which were randomly selected periodically every fortnightly over three months (April – June, 2016) from the respective three markets. Retailers collected the frozen fish from commercial cooling blast frozen fish distribution during 2016 sales batch. Retailers distributed their fish from carton packed containers at ambient temperature.

Collection of Samples

One hundred and eight frozen markerel fish (*Scomber scombrus*) of viable consumable fish food were collected from retail fish sellers into respective sterile polythenes inserted in a cooling bag to prevent external contaminations. The fish were immediately transported to the laboratory where length (cm) and weight (g) of the fish species were determined and bacteriological examinations carried out.

Bacteriological Examinations

Each fish was cut into head, trunk and tail regions with sterile knife on a sterile platform cleaned with methylated spirit. This was done to know the region of the fish where bacterial best prevailed. A total of five hundred and forty-six samples were subjected to replicated bacteriological examinations.

Nutrient agar (NA) and MacConkey (MCA) were prepared according to manufacturer's specifications. Prepared NA agar and MCA agar were sterilized at 121^oC for 15minutes in an autoclave. Cooled agars were poured into sterilized petri-dishes, and examinations were done according to IFAS, (2006). Colonies appeared as clusters and each plate was counted and recorded.

Statistical Analysis

Data collected from samples were analysed using descriptive and analytical methods. T-test was used to analyse bacterial isolated data within location and one-way ANOVA was used to analysed data on bacterial isolated among market locations using SPSS 18. Descriptive statistics were used to show differences in bacterial isolates by market locations and fish parts.

Results

Six bacteria were isolated from the samples and these were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhii*, *Shigella dysenteriae*. *Staphylococcus aureus* was the most dominant bacteria in terms of quantity across the part of the fish examined. The bacteria load was highest in the head of the fish samples examined, while the trunk had the least (Figure 1).

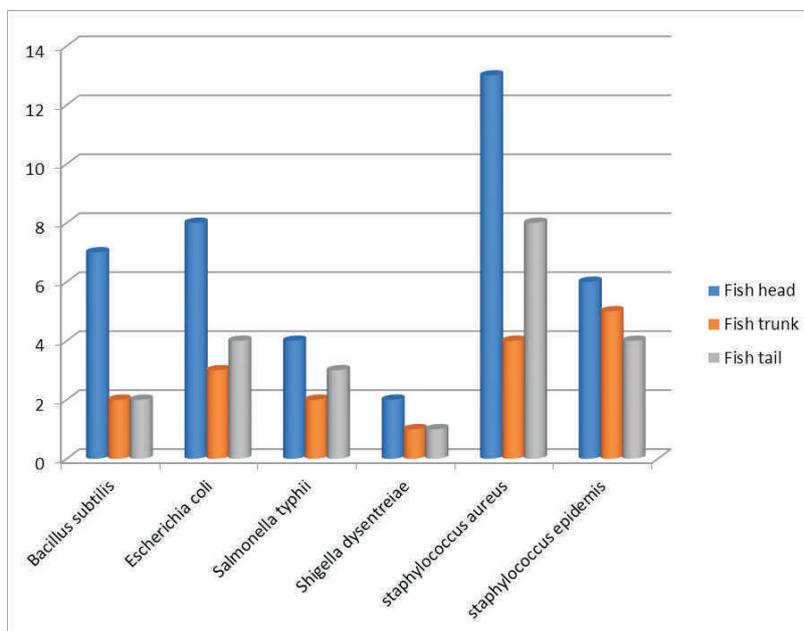


Figure 1: Occurrence of bacteria (cfu/mL) between fish head, trunk and tail of *S. scombus* samples from three markets. (No of fish samples from the three markets: n = 108)

Table 1: Percentage occurrence of bacterial floral isolated from the sampled fish

| Market / stalls | <i>S. aureus</i> (%) | <i>S. epidermis</i> (%) | <i>E. coli</i> (%) | <i>B. subtilis</i> (%) | <i>S.typhii</i> (%) | <i>S. dysenteriae</i> (%) |
|-----------------------|----------------------|-------------------------|--------------------|------------------------|---------------------|---------------------------|
| Oja Oba | 35 | 20 | 15 | 15 | 10 | 5 |
| Isikan | 34 | 23 | 23 | 5 | 10 | 15 |
| Stateline area Stalls | 35 | 25 | 25 | 10 | 15 | 15 |

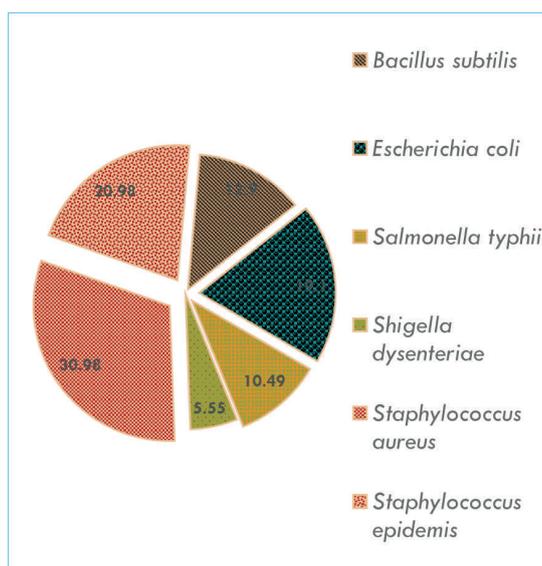


Figure 2: Percentage Bacteria load on the *Scomber scombrus* fish

Bacteria load in Oja – Oba samples ranked highest among other markets; and presence of *Salmonella typhi* and *Shigella dysenteriae* indicate fecal and poor environmental status. *S. aureus* ranked highest in occurrence and *S. dysenteriae* had the least occurrence, (Table 1 and Figure 3).

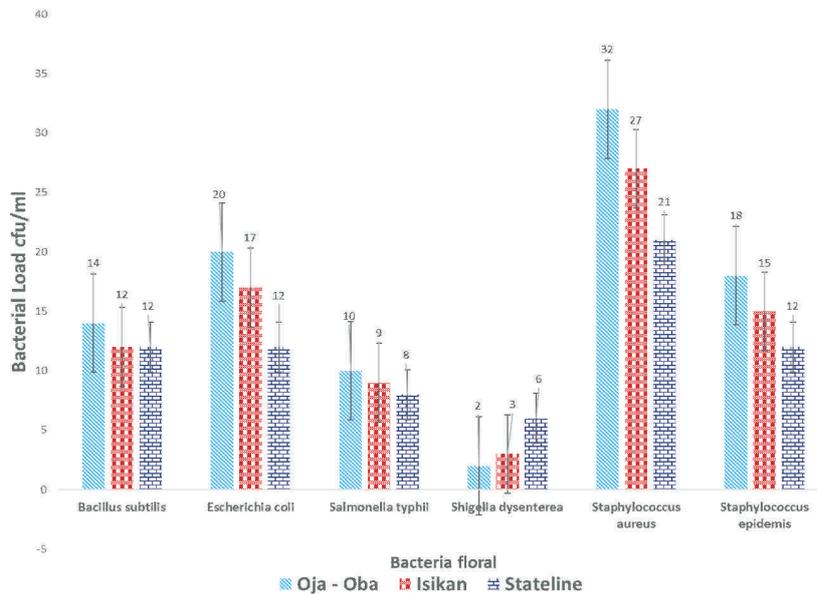


Figure 3: Bacteria load on *Scomber scombrus* fish samples from the market locations

Discussion

The study revealed the presence of different species of bacteria that could be associated with frozen fish. The bacteria isolated in this study are like those reported by Abolagba and Igbinevbo (2010). All the pathogens isolated are of food and public health implication and hence hazardous and injurious to human health if consumed without proper washing and cooking. The report is supported by the work of Ajayi (2011), who isolated bacteria that could cause ill health. The difference in the bacteria counts of samples obtained from the three different locations showed that the quality standard of the product varies and could be as a result of handling techniques; as stated by the work of Moshood and TengkuHaziyaamin (2012) during the handling of fish. The natural flora of fish environment will be contaminated with organisms associated with man which can grow well at 30-37⁰ C temperature range.

The result of this study revealed that *Staphylococcus aureus*, *Shigella* spp., *Staphylococcus epidermis*, *Bacillus subtilis*, *Bacillus subtilis*, *Escherichia coli* were the pathogenic bacteria found associated with frozen fish in Akure, Ondo State. The percentage occurrence of bacteria varies depending on market location as well as fish part location, as highest load was isolated at the head region of the fish. This is in line with the findings of Shinkafi and Ukwaja (2010), who reported occurrence of bacterial in fish parts.

Staphylococcus aureus occurred most among other bacterial floral isolated; and its occurrence is of public health significance because it has been incriminated with food borne intoxication and infection. (Moshood and TengkuHaziyaamin, 2012). This organism could have contaminated the fish through poor human handling.

Staphylococcus epidermis is a gram-positive bacterium and one of the over 40 species belonging to the genus *Staphylococcus*. It is part of human skin flora (commensal), and consequently part of human flora. It can also be found in mucous membranes and in animals. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are often at risk for developing an infection.

Conclusion

The result obtained indicates that most times, frozen fish are preserved under unhygienic environment whereby they are exposed to microorganisms before getting to the final consumers. This lack of proper sanitary in retailed frozen fish handling technique is of public health significance. Therefore, to minimize the risk of infectious disease, it is advisable to follow better hygienic and culinary processes prior consumption.

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