Assessment of the Microbiological Quality of Street-vended Ready-To-Eat Bole (roasted plantain) Fish (*Trachurus Trachurus*) in Port Harcourt Metropolis, Nigeria

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ABSTRACT: The study was carried out to assess the microbiological quality of street-vended ready-to-eat bole (roasted plantain) fish (*Trachurus trachurus*) in Port Harcourt metropolis, Nigeria. Eighteen samples of roasted plantain and fish were obtained from three (3) locations in Port Harcourt and analyzed using standard techniques. Inoculations were done using the spread plate and the Most Probable Number (MPN) techniques. Results showed that the total viable count of the head of the fish ranged from 1.2×10^6 to 5.08×10^6 CFU/g and the mid section ranged from 1.0×10^6 to 2.83×10^6 CFU/g. The percentage of pathogenic bacteria species isolated from the bole and fish samples were; *Staphylococcus aureus* (46.0%), *Bacillus cereus* (30.0%), *Escherichia coli* (13.3%) and *Proteus* sp. (10.0%). Four spoilage moulds were also isolated and they included; *Aspergillus niger* (33.3%), *Apergillus flavus* (33.3%), *Penicillium* sp. (16.7%) and *Neurospora* sp. (16.7%). The microorganisms isolated from the roasted Atlantic Horse Mackerel were most likely to be as a result of cross contamination from improper handling, storage and display, as well as their survival mechanisms during the roasting process. These microorganisms are involved in a number of diseases of public health concern and it is recommended that the relevant Ministries of Health and Environment enforce laws and ensure strict compliance to these laws.
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1. INTRODUCTION

Shiny, as the Atlantic horse mackerel is locally called is barbecued and eaten alongside roasted plantain, bole. Its binomial name is *Trachurus trachurus* and it is specie of the *Carangidae* family. It gets its common name from the legend that other smaller fishes could ride on its back over great distances. Other common names include common scad, maasbanker Pollock, saurel and rough scad. The nutritional value of fish has led to its worldwide acceptance and consumption. Raw fish has become the most susceptible of all food to microbial spoilage as microbes such as fungi, bacteria and viruses are commonly associated with fresh fish (Donnenberg, 2005).

Seafood has traditionally been a popular part of the diet and main supply of animal protein in many parts of the world (Amusan et al., 2010). Fish and fishery products constitute an important food component for a large section of world population, more so in developing countries, where fish forms a cheap source of protein (Amusan et al., 2010). In the last two decades there has been an increase in awareness about the nutritional and health benefits of fish consumption (Amusan et al., 2010). The low fat content of some fish and the presence of polyunsaturated fatty acids in red meat fishes which are known to reduce the risks of coronary heart diseases, have increased the dietary and health significance of seafood consumption (Amusan et al., 2010). The Food and Agriculture Organization (1994) asserted that fish contributes about 60% of the world's supply of protein and that 60% of the developing world derives more than 30% of their annual protein from fish (Amusan et al., 2010). However, in Nigeria, fish constitute 40% of the animal protein intake (Olatunde, 1998; Amusan et al., 2010). They are prone to contamination at various stages of handling and processing and the quality is a major concern to food processors and public health authorities (Oramadike et al., 2010; Amusan et al., 2010).

Street-foods are foods and beverages that are sold by street vendors or hawkers, and the foods and beverages could be raw or cooked (Ameko et al., 2012). The various varieties of street-foods evolve round the common starchy staples of maize, cassava, rice, plantain, and yam; legumes like cowpea and groundnuts; vegetables like tomatoes, onions, pepper, lettuce, spring onions, cabbage, carrots and cucumber; animal protein like goat meat, beef, and various types of fishes (Ameko et al., 2012). The oils are from either palm oil or groundnut oil (Ameko et al., 2012).

Street vending foods are readily available sources of meals for many people but the biological safety of such food is always in doubt (Feglo and Sakyi, 2012). Street foods (ready-to-eat foods sold in the informal sector) form an important and well established sector of the food industry in developing countries, Nigeria inclusive (Adu-Gyamfi and Nketsia-Tabiri, 2007). The street food industry plays a very important role in meeting food requirements of commuters and urban dwellers in many cities and towns of developing countries, as it feeds thousands of people daily with a large range of foods that are relatively cheap, easily accessible (Adu-Gyamfi and Nketsia-Tabiri, 2007; Tambekar *et al.,* 2008; Feglo and Sakyi, 2012), nutritionally-balanced and also provide a source of income for the vendors (Swanepoel *et al.*, 1995; Adu-Gyamfi and Nketsia-Tabiri, 2007). Despite these benefits, concerns have been raised about their safety and quality because most of the vendors lack training in basic food safety practices concerning raw material acquisition, food preparation, storage, handling, and final delivery to the consumer (Adu-Gyamfi and Nketsia-Tabiri, 2007). However, food borne illnesses of microbial origin are a major health problem associated with street foods (Mensah *et al.,* 2001, 2002; Adu-Gyamfi and Nketsia-Tabiri, 2007; Feglo and Sakyi, 2012).

The popular method of preparation is mainly by salting the fish after washing, arranged on the smoking tray and then smoked in the charred coal fire in their residence. No information concerning the safety of this type of street-vended smoked-fish is available. Researchers who investigated the microbiological quality of street-vended foods have reported high bacterial counts and a high incidence of food borne pathogens in such foods in different countries (Bryan et al. 1992). The number of people suffering from food borne illness has increased dramatically over the last decade. From the standpoint of microbiology, fish and related products are a risky foodstuff group. Particularly, *Clostridium botulinum* type E and *Vibrio parahaemolyticus* rank among pathogenic bacteria associated with fish. Other potentially pathogenic bacteria associated with fish and shellfish include *C. perfringens*, *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *V. cholerae* and other vibrios. Outbreaks usually occur due to the ingestion of insufficiently heat-treated fish or products contaminated after or during their processing. Freezing fish and related products in the seawater, poor handling, long-time transport or cooking in fishing containers straight on the deck contributes to their contamination with microorganisms (Novotny et al., 2004).

The traditional processing methods that are used in the preparation, inappropriate holding temperature and poor per-sonal hygiene of food handlers are some of the main causes of contamination of ready to eat foods (Mensah *et al.,* 2002; Barro *et al.,* 2006; Feglo and Sakyi, 2012). Also these foods are not effectively protected from flies and dust (Bryan *et al.,* 1992, 1997; Feglo and Sakyi, 2012). The study was carried out to assess the microbiological quality of street-vended ready-to-eat bole fish (*Trachurus trachurus*) in Port Harcourt metropolis, Nigeria.

2. MATERIALS AND METHODS

The major material used for the analysis was roasted Atlantic horse mackerel (*Trachurus trachurus*) popularly known as "Bole fish" and the fresh one that has not been roasted. All samples were obtained from Uniport, GRA and homemade, which are all located in Port Harcourt metropolis; other materials used during the analysis include; Stomacher blender (steward, model: stomacher 400, UK), stomacher bags and Autoclave (Dixon's Model: ST 19E,UK). The media for isolation were Nutrient agar (NA 7, Flaka Analytical), Mannitol Salt Agar (MSA) (Prona disa), MacConkey agar (Fluka Analytical), MacConkey Broth (Fluka Analytical), Sabouraud Dextrose Agar (SDA), Triple Sugar Iron Agar (TSI), Simmon's Citrate Agar and Eosine Methylene Blue agar, Xylose Lysine Deoxycholate (XLD) medium and buffered peptone water.

2.1. SAMPLE PROCUREMENT

2.1.1. Street vended samples

Eighteen samples of the barbecued fish were obtained from the food handler immediately after procurement from 3 locations in Port Harcourt to represent the different population densities in Port Harcourt. Location A was the University of Port Harcourt which represented a high population density area, Location B was at G.R.A which represented a low population density area and Location C was the homemade samples. The street vended samples were collected into polyethylene bags and the collection was by direct introduction of the fish samples into the bags with minimum exposure to avoid contamination.

2.1.2. Homemade samples

Fish preparation

The fish is purchased from the market, taken t the house and gutted on a clean chopping board. The fish is then washed with clean water until traces of fresh blood is removed.

Fish paste preparation

The key ingredient in the processing of the fish is the fish paste. It comprises dry pepper, palm oil and seasoning powder. These ingredients are homogenized manually using a spoon or spatula until

distribution of the various ingredients is uniformly spread.

Barbecued fish preparation

The fish is soaked in the slurry for about 30 minutes to 1 hour and they are removed and carefully spread out on a clean tray. The tray is covered with a net to keep flies from perching on the fish. The fish is then roasted for about 7– 10 minutes and is ready for consumption with the roasted plantain and the hot palm oil sauce.

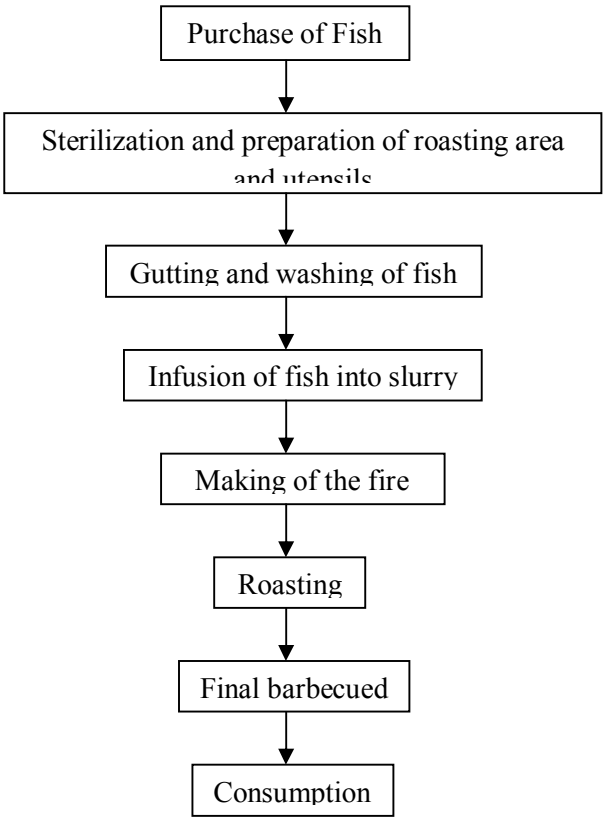

Figure 1: Flow Chart of the barbecued fish production

2.2. Enumeration of micro-organisms

Twenty five grams of the sample was weighted into a sterile stomacher bag containing 225ml of buffered peptone water. The bag was placed in the stomacher blender (steward, model: stomacher 400, UK) and the sample was allowed to blend at 260rpm (revolution

per minutes) for 2 minutes. After blending, serial dilution was carried out to 10^{-3} using the stock as $10^{-1.}$ Appropriate serial dilutions of all the samples were carried out and 0.1ml each of selected dilutions was plated using the pour plate method (Harrigan and McCance, 1976). Enumeration of total aerobic viable count was done using plate count agar (Oxoid, CM325, UK). Eosin methylene blue (EMB) agar (Oxoid) was used for coliform count and Baird Parker agar (Oxoid) supplemented with tellurite and egg yolk emulsion for Staphylococcal counts. Yeast and mould counts were done on Sabouraud dextrose agar (Oxoid). All cultures were incubated at 37^{O}C for 24h except for coliform organism which was incubated at 37^{O}C and 44^{O}C for 24h while yeasts and mould counts was incubated at 25^{O}C for 72 h. All media used were prepared according to the manufacturers' instructions.

2.3. Characterization of isolates

Confirmation of coliform organisms were carried out by inoculating colonies into lactose broth with Durham tubes and incubating at 37^{O}C and 44^{O}C for 24h and another 24h in the absence of gas production (Speck, 1976). The presence of gas constituted a presumptive test and the broth was streaked out on EMB agar incubated at 37^{O}C for 42h. Typical colonies on EMB plates appearing bluish black with greenish metallic sheen which are characteristics of *E. coli* or brownish colonies often convex and mucoid which are characteristics of *Enterobacter aerogenes* confirmed the presence of coliform organisms. Isolates were stored on nutrient agar slants at 4^{O}C for further confirmatory tests which included IMVIC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, nitrate reduction, urease production and motility. Large, flat, irregular, wrinkled or smooth, ground-glass colonies, 4–6mm in diameter were counted as *Bacillus*. Confirmation was as described by Yusuf *et al.* (1992). Confirmation of typical colonies of *S. aureus* on Baird–Parker agar was on the basis of the results of catalase, coagulase, phosphatase production, nitrate reduction and carbohydrate utilization (Umoh *et al.,* 1999). For isolation and confirmation of *Salmonella* and *Shigella*, procedures recommended by Speck (1976) were followed. The pre-enriched samples in lactose broth were subcultured into selenite F broth for selective enrichment, and on Salmonella–Shigella agar (SSA). Typical colonies were Gram-stained and characterized (Speck, 1976).

2.4. Statistical analysis

One-way analysis of variance and least significance difference (LSD) were used to compare means of isolates obtained from the various samples analysed.

3. RESULTS ANALYSIS

3.1. Enumeration of isolates

The total viable count for aerobic mesophilic bacteria of the head of barbecued Atlantic Horse Mackarel was 7.8×10^{6} CFU/g (Location A), while the

middle had 5.5×10^5 CFU/g (Location A). The rest of the results are presented in Table 1.

Table 1: Microbial loads

Location	Total coliforms count (CFU/g)	Total viable count (CFU/g)	Total *Staphylococci* count (CFU/g)	Total Fungal count (CFU/g)
Location A				
1(Head)	$1.56x10^3$	7.8×10^6	6.56×10^5	0.38×10^5
2(Middle)	$1.1x10^3$	5.5×10^5	5.5×10^5	0.28×10^5
Location B				
1(Head)	$1.2x10^2$	3.25×10^5	3.52×10^5	0.2×10^5
2(Middle)	$3.5x10^2$	3.83×10^6	4.4×10^5	0.28×10^3
Location C				
1(Head)	$2.3x10^2$	3.0×10^5	3.4×10^6	0.23×10^6
2(Middle)	$0.91x10^2$	1.67×10^5	2.87×10^5	0.15×10^5

Key: MPN-Most Probable Number; CFU-Colony Forming Unit

3.2. Isolation and identification of isolates

From the analysis of bole fish (Atlantic horse mackerel), four (4) bacterial species were isolated. They included; *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and *Proteus* sp. In addition to the bacterial species, four (4) fungal species were also isolated, which included; *Aspergillus niger, Apergillus flavus, Penicillium* sp. and *Neurospora* sp.

3.3. Frequency of Occurrence of Bacterial Isolates in relation to Locations

Table 2 shows the frequency of occurrence of bacterial isolates in relation to locations. It showed that *Staphylococcus aureus* (46.7%) was most predominant while *Proteus* sp. (10.0%) was least predominant. *E. coli* and *Proteus* sp. was not present in samples from location C (Table 2).

Table 2: Frequency of Occurrence of Bacterial Isolates in relation to Locations

Isolates	No. (%)	Location A (%)	Location B (%)	Location C (%)
Bacillus cereus	9(30.0)	5(55.5)	2(22.2)	2(22.2)
Staphylococcus aureus	14(46.7)	5(35.7)	5(35.7)	4(28.6)
Escherichia coli	4(13.3)	3(75.0)	1(25.0)	0(0.0)
Proteus sp.	3(10.0)	2(66.7)	1(33.3)	0(0.0)
Total	30(100.0)	15(50.0)	9(30.0)	6(20.0)

3.4. Frequency of Occurrence of Fungal Isolates in relation to Locations

Table 3 shows the frequency of occurrence of fungal isolates in relation to locations. It showed that *Aspergillus niger* (33.3%) and *Apergillus flavus* (33.3%) were most predominant while *Penicillium* sp. (16.7%) and *Neurospora* sp. (16.7%) were least predominant (Table 3).

Table 3: Frequency of Occurrence of Fungal Isolates in relation to Locations

Isolates	No. (%)	Location A (%)	Location B (%)	Location C (%)
Aspergillus niger	10(33.3)	7(70.0)	2(20.0)	1(10.0)
Apergillus flavus	10(33.3)	5(50.0)	2(20.0)	3(30.0)
Penicillium sp.	5(16.7)	3(60.0)	1(20.0)	1(20.0)
Neurospora sp.	5(16.7)	2(40.0)	2(40.0)	1(20.0)
Total	30(100.0)	17(56.7)	7(23.3)	6(20.0)

4. DISCUSSION

In this study, an assessment of the microbiological quality of street-vended ready-to-eat bole (roasted plantain) fish (*Trachurus trachurus*) in Port Harcourt metropolis, Nigeria was carried out. Ready-to-eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Ready-to-eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002). Different terms have been used to describe such ready to eat foods. These include; convenient, ready, instant and fast foods. Examples of such ready to eat foods include; pastries, meat-pie sausage rolls, burger, moi-moi, salad or cole-slaw, roasted fish-fried meat, fried chicken, milk and milk products.

As earlier mentioned; street foods (ready-to-eat foods sold in the informal sector) form an important and well established sector of the food industry in developing countries, Nigeria inclusive (Adu-Gyamfi and Nketsia-Tabiri, 2007). A general observation of our society shows a social pattern characterized by increases in mobility, large itinerary workers and less family or home centered activities. This situation however, has resulted in more street-vended ready-to-eat foods taken outside home. Thus, food vendors services became on the increase and responsibility for good manufacturing practices of food such as good sanitary measures and proper food handling have been transferred from individuals/families to the food vendors who rarely enforce such practices (Musa and Akande, 2002).

Food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. Data on issues of food borne diseases are well documented worldwide (Hazariwala *et al.,* 2003). Food borne illness is a major international health problem with consequent economic reduction (Duff *et al*., 2003).

Street vended foods are important source of nutrients to low income populations groups in developing countries (Amusan et al., 2010). This emphasizes the necessity of organizing this sector to improve its low hygienic and hazardous situations (Amusan et al., 2010). However, it was recognized from this study that some vendors prepared and smoked in poor hygiene conditions, the study confirmed that the sanitary level in the sale places are deteriorated, leading to the occurrence of severe public health hazards (Amusan et al., 2010). Seafood could become a source of bacterial pathogen by exposure to contaminated water or through processing practices thus representing a public health hazard (Iwamoto *et al.*, 2010; Amusan et al., 2010). As observed in the study, the total viable count was 7.8×10^6 CFU/g (Location A), while the middle had 5.5×10^5 CFU/g (Location A). According to ICMSF (1986), the plate count should be less than 10^6 and the borderline limit of acceptability is between 10^6 and less than 10^7. This result is within the acceptable limit (Amusan et al., 2010).

In this study, four (4) bacterial species were isolated from bole fish (Atlantic horse mackerel) which included; *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and *Proteus* sp. In addition to the bacterial species, four (4) fungal species were also isolated, which included; *Aspergillus niger, Apergillus flavus, Penicillium* sp. and *Neurospora* sp. *Staphylococcus aureus* (46.7%) was most predominant while *Proteus* sp. (10.0%) was least predominant. *E. coli* and *Proteus* sp. was not present in some of the locations. Outbreaks of food borne diseases are caused by foods that are contaminated during harvest, processing or preparation. In most countries, the most common food borne illness is *Staphylococcus* food intoxication (Talaro and Talaro, 1996). *Enterotoxigenic Staphylococcus* strains and *Escherichia coli* strains have been isolated from foods implicated in illness (Cencil *et al.,* 2003). *Escherichia coli* and *Staphylococcus aureus* are normal flora in humans and animals, their presence in foods are indications of excessive human handling (Adamolekun and Adamolekun, 1992).

In this study, samples were obtained from a high population density area, a low population density area and the home made samples. They were tagged location A, location B and location C respectively. Both gram positive and gram negative bacteria were isolated. The total viable count for aerobic mesophilic bacteria from the analysis was; 7.8×10^6 CFU/g (Location A), while the middle had 5.5×10^5 CFU/g (Location A). This fell within the International Committee on the microbiological Specification of Foods (ICMSF, 1986) for roasted or smoked fish, which is 5.0×10^5 to 10^7. In a study by Amusan et al. (2010), the microbial analysis revealed total plate count from $1.9x10^4$ to $7.8x10^5$ Cfu/g from unwashed fish samples, $2.4x10^3$ to $2.06x10^6$ Cfu/g from fish washed with cold water, no organism detected from fish washed with hot water and $1.7x10^5$ to $2.9x10^6$ Cfu/g from fish washed with salt in cold water. Information on street foods in developing countries is not readily available. However, studies on street-vended foods in USA, Asia, and a few African countries have revealed high bacterial counts and presence of foodborne bacterial pathogens (Bryan *et al.*, 1997; Mosupuye and von Holy, 1999; Adu-Gyamfi and Nketsia-Tabiri, 2007). Aerobic mesophilic count (AMC) exceeding 4×10^5 CFU/g, *Staphylococcus aureus* count exceeding 3×10^4 CFU/g and *Bacillus cereus* count exceeding 2×10^5 CFU/g have been reported for vegetable salads and pepper sauce served with street foods in most developing countries; Ghana and Nigeria inclusive (Adu-Gyamfi and Nketsia-Tabiri, 2007; Odu and Ameweiye, 2013). According to the World Health Organisation, effects of microbiological hazards such as *Salmonella, Campylobacter jejuni* and enterohaemorrhagic *Escherichia coli* on food safety is now a major public health concern worldwide (WHO, 2004; Adu-Gyamfi and Nketsia-Tabiri, 2007).

The most predominant bacterial pathogens isolated in the present study include *S. aureus*, *Bacillus cereus*, *E. coli* and *Proteus* sp. The isolation of these pathogens has also been reported by previous workers from various foods (raw and ready-to-eat foods) (Fang et al., 1999). A study on street vended foods in Atbara City in the Naher Elneen state of Sudan showed that the most prevalent bacteria contaminating cooked meals, bottled drinks and fresh fruit *were Escherichia coli, Staphylococcus aureus* and *Bacillus* sp. (Abdalla et al., 2009; Elfaki and Elhakim, 2011; Ameko et al., 2012). The magnitude of food-borne contamination in bole fishes tested indicated for the wide prevalence of food-borne diseases. Therefore, the detection of these food-borne pathogens in high numbers from bole fishes of various origins is an indication of poor hygienic practices in the study area.

Staphylococcus aureus (46.0%), been the most predominant in this study was isolated from some fish samples in all locations (A-C). It is not surprising in such poor hygiene and handling

situations to isolate *Staphylococcus* spp from the majority of samples, a well-known food-borne pathogen, which rarely has been implicated in cases originating from consumption of smoked sea foods (Amusan et al., 2010). This bacterium may be contributed through human handling of the raw seafood and products. Nevertheless, adequate precautions can prevent *S .aureus* contamination, growth and enterotoxin production from occurring in smoked fish products (Himelbloom et al., 2008; Amusan et al., 2010). Contamination of ready-to-eat products can be prevented through the use of latex gloves to reduce excessive human hand contact (ICMSF, 2000; Amusan et al., 2010). Open-air markets have been implicated in direct transfer of *S. aureus* during handling between traders and customers of ready-to-eat cooked, smoked, dried, or fried fish and shellfish (Amusan et al., 2010).

Staphylococcus aureus is a gram positive coccus resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non-pathogenic. They produce some enzymes which are implicated with *Staphylococcal* invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal (Prescott *et al.,* 2005). Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene-based that is carried on plasmids. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Some signs and symptoms of *Staphylococcal* food poisoning include nausea, vomiting, abdominal cramp and diarrhoea (Amusan et al., 2010).

Bacillus sp (30.0%) was second predominant bacterial isolates in this study. This consistent with what was earlier reported by Odu and Ameweiye (2013). *Bacillus sp.* produces toxins that withstand high temperatures and are spore forming which germinate and release enterotoxins. Ingestion of bacillus toxin- containing food also causes nausea, vomiting, abdominal cramps and diarrhea (Adebayo-Tayo et al., 2006, 2009, 2012a,b; Kapute et al., 2012). Since some species of *Bacillus* are airborne and dust- borne contaminant, exposing of the roasted fish and poor handling can lead to contamination (Odu and Ameweiye, 2013).

Contrary to what was earlier reported by Odu and Ameweiye (2013). *Escherichia coli* (13.3%) was the third predominant bacterial isolates. The isolation of *Escherichia coli* from the samples of bole fishes poses food safety problems since they are all enterotoxigenic and cause gastroenteritis (Adu-Gyamfi and Nketsia-Tabiri, 2007). The presence of *E. coli* in bole fish reflects secondary contamination, as *E.coli* is known to be associated with the gastrointestinal tract of warm-blooded animals, and not known to be present in the environment as a natural flora (Amusan et al., 2010). Sewage contamination of fish harvesting areas is the major reason for the presence of *E. coli*, but contamination can occur through the use of non-potable water or ice in the landing centers or fish markets (Kumar *et al*., 2004). *E. coli* has been reported in foods sold at cafeteria in a study by Alyaaqoubi et al. (2009). Our results were not comparable to Meldrum et al. (2006), who found no *E. coli* in RTE burgers, pasty meat and pate meat in the UK.

E. coli was present in some of the locations except location C. *Escherichia coli* is a member of the genus *Enterobacteriaceae*. Members are widely distributed in the environment. Contaminated food and water are the major sources by which the bacterium is spread. Selected strains can cause a wide variety of infections in hospitals and community settings (Donnenberg, 2005). These include diarrheal illness, urinary tract infections, meningitis, sepsis, wound infections, nosocomial pneumonia and dysentery. A subgroup called Enterohaemorrhagic *Escherichia coli* (EHEC) can cause food borne illness as the *Escherichia coli* 0157:H7 strain which cause severe and potentially fatal illness known as Haemorrhagic colitis which is characterized by bloody diarrhea and severe abdominal pain (Dolores and Doyle, 2001). *Escherichia coli* is commonly used as a surrogate indicator; its presence in food generally indicates direct or indirect fecal contamination. However, in Nigeria, a number of foods have been reported to have a high incidence of the bacteria (Adeyisun, 1995; Okonko *et al.,* 2009). But there is limited information on the health challenges from ready to eat foods vended in the Port Harcourt metropolis. Therefore, it is important that all stages of fish production, handling and processing are monitored for *E. coli* contamination (Amusan et al., 2010).

Contrary to what was earlier reported by Odu and Ameweiye (2013), *Klebsiella* spp. was not found in any fish samples from the three locations. Bacteria that belong to the genus *Klebsiella* are facultative, anaerobic, non motile, Gram-negative rods that possess a prominent polysaccharide capsule (Umeh and Berkowitz, 2009; Odu and Ameweiye, 2013). *Klebsiella* species exist as normal flora in the gastrointestinal tract of animals and humans (Siri et al., 2011; Odu and Ameweiye, 2013). Despite this, *Klebsiella* species can cause severe infections that include meningitis, bronchitis, bacteremia, pneumonia, urinary tract infections in humans and animals (Lau et al., 2007; Siri et al., 2011). In

humans these infections are common in patients who are admitted in hospitals and those who are immunocompromised (Siri et al., 2011). Thus most infections caused by *Klebsiella* species result from consumption of contaminated food such as rotten fish and/or water (Haryani et al., 2007; Siri et al., 2011). An incident of *Klebsiella pneumonia*e has been reported in street foods sold in Malaysia (Haryani et al. 2007; Alyaaqoubi et al., 2009).

In agreement with what was reported by Soriano et al. (2002) in Spain, Alyaaqoubi et al. (2009) in Malaysia and Odu and Ameweiye (2013) in Nigeria, no *Salmonella* spp was found in this study. However considering the low sample size together with the negative data, it is not claimed that street-vended ready-to-eat bole fishes are free from *Salmonella* spp rather it does indicate that the prevalence of *Salmonella* in street-vended ready-to-eat bole fishes is very low (Rabbi et al., 2011). Other studies have identified *Salmonella* spp. on other street foods and their accompaniments in South Africa (Adu-Gyamfi and Nketsia-Tabiri, 2007) and Zambia (Bryan *et al.*, 1997; Adu-Gyamfi and Nketsia-Tabiri, 2007). While *Salmonella* sp. causes salmonellosis and typhoid fever, *E. coli* O157:H7 causes severe illness and deaths, especially among children in several countries (Adu-Gyamfi and Nketsia-Tabiri, 2007).

The spoilage moulds were *Aspergillus niger, Apergillus flavus, Penicillium* sp. and *Neurospora* sp. These fungal pathogens have been reportedly isolated from different sources in Nigeria (Chukwuka et al., 2010; Al-Hindi et al., 2011; Akintobi et al., 2011; Adebayo-Tayo et al., 2012a,b,c,d; Odu and Ameweiye, 2013). The most common fungi found in a study by Akintobi et al. (2011) were *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and yeasts. The group of diseases caused by Aspergillus are called aspergillosis, the symptoms include fever, cough, chest pain or breastlessness. Usually, only patients with already weakened immume systems or who suffer other lung condition are susceptible (Adebayo-Tayo et al., 2012b; Odu and Ameweiye, 2013).

In this study, *Aspergillus niger* (33.3%) and *Aspergillus flavus* (33.3%) were the most predominant fungal isolates. A similar trend was also reported by Adebayo-Tayo et al. (2012a,b) in their study on fishes. The various moulds observed can cause various human diseases, and are encouraged by exposure of the roasted fish to humidity. *Aspergillus flavus* produces toxins also known as aflatoxin which causes mycotoxicosis which can also lead to liver cancer in humans, cirrhosis and algaltocosis/hepatitis (Odu and Ameweiye, 2013). The presence of *Aspergillus* in this study reveals possible production of aflatoxins (Adebayo-Tayo et al., 2006, 2012a,b,c,d; Odu and Ameweiye, 2013).

In this study, *Penicillium* sp. (16.7%) and *Neurospora* sp. (16.7%) were least predominant fungal isolates in this study. Contrary to this finding, Adebayo-Tayo et al. (2012c) reported *Penicillum* spp. predominant over *Aspergillus spp*. in their study. In another study by Adebayo-Tayo et al. (2012c) on tilapia fish, *Aspergillus sp* and *Penicillium sp* (16.7%) was reported to be least predominant. *Penicillium* sp. also produces mycotoxins that are harmful to man and may result in renal damage/necrosis of the kidney while *Neurospora* sp. produce spores that may cause asthma (Odu and Ameweiye, 2013). Generally, the mould observed is pathogenic and cause harm to humans (Adebayo-Tayo et al., 2012c,d).

It should be noted however, that the organisms isolated from the samples were pathogenic organisms that could become harmful and cause food borne intoxication. Location A, which represents the high population density area, was observed to produce a larger number of bacterial contaminations compared to population B which is a low population and high income earning area. It was noted that the head of the fish had a higher bacterial load than the middle. This could be attributed to the presence of gills in the head of the fish which they use for feeding and could serve as a means of attachment to microorganisms. The variance in the microbial load can also be attributed to a higher number of vehicular and human movements, dust settlement on the fish and level of exposure of the fish to the environment. In comparing the microbial load of the barbecued fish to the raw fish, it was observed that the barbecued fish contained a higher number of microorganisms than the roasted fish, even after the roasting process. The explanation for this could be that cross examination occurs from the food handler either from the practice of improper sanitation or from the spices that are used in seasoning of the fish. The women vendors lack good personal hygiene, which is vital in reducing the chance of contamination of foods. It is not surprising in such poor hygiene and handling situation to isolate *Staphylococcus* spp. from the majority of samples (Amusan et al., 2010). To minimize health risks associated with this type of food, it is important to organize the basic food hygiene training for the women vendors of bole fish in Port Harcourt metropolis, Rivers State, Nigeria and also educate the consumers on the soaking of smoked fish for about 30 minutes in boiled water before consumption to avoid food poison (Amusan et al., 2010).

The microbiological quality and safety of ready-to-eat foods are also influenced by processing steps

and storage conditions that introduce other microorganisms. It has also been observed that many people buy the roasted fish and keep it for a while before consumption, thus, encouraging the growth of microorganisms which may cause food poisoning on consumption. It is therefore suggested that the fish be consumed immediately after purchased to reduce the risk of food poisoning to a minimum. This study shows that the handling, storage and display of the fish encourage cross-contamination and re-contamination of the fish. Some measure can be carried out to curtail the risk contamination during preparation or consumption of the fish. Provision of information on the health implications of pathogens introduced during cross contamination or re-contamination. Reported information in this study could be used to improve handling and preparation of this nutritious fish and protect its ever-increasing consumers. It can also be a source document for further studies and useful for public education on fish. Therefore smoking of fishes requires more attention from health authorities, educational programs for vendors and improvement of preparation and handling environments.

5. CONCLUSION

Ready to eat foods contain the indigenous microflora of the raw materials from which they are prepared. Pathogens may form part of the microflora, posing a public health problem. Pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *Proteus* sp., *Aspergillus niger, Apergillus flavus, Penicillium* sp. and *Neurospora* sp. were the main identified organisms in bole fishes which are the most common diseases reported from the health sector of most regions in Africa. Thus, health hazards from bole fishes may be minimized by avoiding poor handling and awareness of personal hygiene and care in preparation, storage and dispensing of bole fishes in all procedures necessary to maintain the safety and suitability of bole fish from the locations, periodic sanitary-hygienic evaluation and inspection of street-vended ready-to-eat foods should be strengthened to reduce public health hazards associated with food-borne pathogens.

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