Microbiological Quality Of Street-Vended-Ready-To-Eat "Bole" Fish In Port Harcourt Metropoplis

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ABSRACT: Fish, a staple food is readily contaminated with microorganisms. This study was carried out to analyze the possible microorganism that are of public health concern in connection with roasted fish. A total of 18 samples were obtained from Ozuoba, Rumuokoro and Ada-George in Port Harcourt and plated on different media. The samples were analysed using the spread plate and MPN techniques. The percentage occurrences of the bacteria were *Staphylococcus aureus* (35.7%), *E. coli* (17.9%), *Klebsiella sp* (21.4%), and *Bacillus sp* (25.0%) while fungal isolates were *Neurospora* sp (16.7%), *Aspergillus flavus* (25.0%), *Aspergillus niger* (41.7%) and *Penicillium* sp (16.7%). The total viable counts of the street-vended ready-to-eat bole fishes ranged from $2.8x10^3$ to $6.3x10^5$ cfu/g. Ready-to-eat bole fishes sold in the streets of Port Harcourt had the highest number of total coliforms (1560 MPN/g) with an average of 1100 MPN/g at the second sampling of the vending day. Aseptically homemade preparation of the roasted fish showed low microbial load. The study showed that the locations of roasted fish; handling and the personal hygiene of the vendors affect the microbial load of the roasted fish. Therefore, this study has shown that despite the heating of the fish, microorganism was still observed. However, based on the acceptable level of the microbiological guideline/standards, the total viable count, Staphylococcus count, and total coliform counts were exceeded in all samples collected at intervals of marketing times. Good personal hygiene was recommended.
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1. INTRODUCTION

Street vended foods are defined as those foods prepared on, the street and ready to eat, or prepared at home and consumed on the street without further preparation (Bryan et al., 1997). Street-vended foods include foods as diverse as meat, fish, fruits, vegetables, grains, cereals, frozen produce and beverages (World Health Organization (WHO), 1996). Each year, millions of people worldwide suffer from food-borne diseases (WHO, 2000), and illness resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society (Notermans *et al.,* 1995). Street foods, particularly in developing countries, have been reported to be contaminated by pathogenic bacteria (Arambolu *et al.,* 1993). There are increased interests worldwide on the importance of street food as part of general concern for food security and health (Canet and N'Diaye, 1996). The incidence f food-home diseases is rising in developing countries, as well as in the developed world (Raymond and Griffith, 2003).

In several developing countries including Nigeria, the statistics on the incidence of food borne diseases are not available, however, the high prevalence of diarrheal diseases, particularly in infants and young children in these countries is an indication of an underlying safety problem (Omemu and Omeike, 2010). Although the precise extent of food borne diarrhoea diseases in young children is not documented, but a review by Esrey and Feachem (1989) concluded that indirect evidence suggested that 15% to 70% of all diarrheal episodes may be associated with practises of food preparation, handling and storage as well as feeding methods.

Street foods are part of catering business in developing countries, particularly in urban areas. Most of these products are ready-to-serve or ready-to-eat foods sometimes under poor cooking and trading conditions which can lead to poor nutritive value and low hygienic quality. Food sold in the streets are very common in developing countries. Prepared, most often by women as commercial activities, they have the advantage to be varied. We found meat, fish, cereals, milk, etc. and available at all time (Obayelu *et al*., 2009). As such, this consuming mode is the mode of quick and easy consumption affecting all social groups (Mensah *et al*., 2002).

The main factors which determine food hygiene include handling, preparation techniques and storage practices (Ifediora, *et al.* 2006). In Nigeria, not much work has been done to try and investigate the occurrence of pathogenic microorganisms in cooked *ogi* taking into consideration the method of

preparation and storage practices. However, the conditions of preparation and selling are sources of contamination especially by germs involved in poisoning food (Bukar *et al*., 2010). Diseases resulting are a major problem of food safety and a reason of mortality in developing countries (Bukar *et al*., 2010). Faced with this issue of public health in development countries (Elmahmood and Doughari, 2007), the aim and objective of this study was to assess the microbial flora of Atlantic horse mackerel (*Trachurus trachurus*) in different areas in Port Harcourt metropolis, to highlight the possible microorganisms that can affect fish and they can be identified, to suggest possible remedies for the effects of the microbial flora of Atlantic horse mackerel (*Trachurus trachurus)*, to identify microorganisms of public health concern and to make possible recommendations to processors and consumers.

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 Ozuoba, Rumuokoro and Ada-George axis, 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 Broth (Fluka Analytical), Sabouraud Dextrose Agar (SDA), Triple Sugar Iron Agar (TSI) and Simmon's Citrate Agar.

2.1. Preparation of the Home Made Roasted Fish

The raw fish was washed thoroughly especially the head region to remove blood and other dirt, the you prepare your sauce by mixing pepper, seasoning and salt in red oil and rub the raw fish, then allow for some time to enable the sauce to sink inside, then you roast by placing it on top of a wire gauge under red hot charcoal and allow to roast. The fish with rubbed with the sauce and placed on the wire gauge and allowed to roast.

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 at260rpm (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

From the study, four bacteria were isolated which includes, *Klebsiella sp., E. coli, Staphylococcus aureus, Bacillus cereus* while four (4) fungi were isolated and they include *Neurospora sp, Penicillium sp, Aspergillus niger, and Aspergillus flavus.* Table 1 shows the microbial load for roasted fish

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	Total Staphylococcal count (CFU/g)				**Total viable count (CFU/g)**				**Faecal coliform (MPN/ml)**	
Fish type/parts	Head		Middle		Head		Middle		Head	Middle
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Fresh Atlantic Horse Mackerel	$2.8x10^3$	$1.9x10^5$	$2.2x10^3$	$1.6x10^5$	$6.0x10^3$	$4.3x10^5$	$5.7x10^3$	$4.0x10^5$	43	43
Street Vended Roasted	$3.6x10^3$	$2.3x10^5$	$2.8x10^3$	$1.3x10^5$	$8.0x10^3$	$6.3x10^5$	$5.7x10^3$	$3.8x10^5$	1560	1100
Aseptically Prepared	$1.9x10^3$	$1.3x10^5$	$1.6x10^3$	$1.1x10^5$	$4.3x10^3$	$3.9x10^5$	$4.0x10^3$	$3.5x10^5$	7.3	7.3

Table 2 shows the frequency of occurrence of bacterial isolates. It showed that *Staphylococcus* sp was most predominant (35.7%) while *E. coli* was least predominant (17.9%).

Table 2: Frequency of occurrence of bacterial isolates

Bacterial isolates	**No. (%)**	Fresh Atlantic Horse Mackerel fish (%)	Street Vended Roasted fish **(%)**	Aseptically Prepared fish **(%)**
E. coli	5(17.9)	3(60.0)	2(40.0)	0(0.0)
Staphylococcus sp	10(35.7)	4(40.0)	3(30.0)	3(30.0)
Klebsiella sp	6(21.4)	2(33.3)	2(33.4)	2(33.3)
Bacillus sp	7(25.0)	4(57.1)	2(28.6)	1(14.3)
Total	**28(100.0)**	**13(46.4)**	**9(32.1)**	**6(21.4)**

Table 3 shows the frequency of occurrence of fungal isolates. It showed that *Aspergillus niger* was most predominant (41.7%) while *Neurospora* sp and *Penicillium* sp were least predominant (16.7%).

Table 3: Frequency of occurrence of fungal isolates

Fungal isolates	**No. (%)**	Fresh Atlantic Horse Mackerel fish (%)	Street Vended Roasted fish **(%)**	Aseptically Prepared fish **(%)**
Neurospora sp	2(16.7)	1(60.0)	1(40.0)	0(0.0)
Aspergillus flavus	3(25.0)	1(40.0)	2(30.0)	0(30.0)
Aspergillus niger	5(41.7)	2(33.3)	2(33.4)	1(33.3)
Penicillium sp	2(16.7)	0(57.1)	1(28.6)	1(14.3)
Total	**12(100.0)**	**13(23.1)**	**9(23.1)**	**6(23.1)**

4. DISCUSSION

Ready-to-eat (RTE) foods included snacks may also be prepared and served separately, including dried meat, fish and cereal based ready-to-eat food (Gadaga et al. 2007; Alyaaqoubi et al., 2009). Several studies conducted to assess the quality of different street foods in several countries have shown that these foods were positive vectors of food borne illnesses (Umoh and Odoba, 1999; Omemu, *et al.* 2005). Several studies showed that different pathogens have been isolated from RTE foods including verocytotoxigenic *Escherichia coli* (VETC), *Salmonella* spp. and *Campylobacter* spp., confirming the risk posed by consuming these foods (Gibbons et al. 2006; Alyaaqoubi et al., 2009). Ingestion of foods containing these types of microorganism causes food poisoning which is a health problem that may significantly reduce economic growth (Alyaaqoubi et al., 2009).

Many studies were done for microbiological quality and food safety evaluation on the RTE foods whether at street vending, restaurants or in specific locations such as hospitals, universities, school and others (Alyaaqoubi et al., 2009); similarly, street food vendors are important factor in food borne infection. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with foods and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer (Omemu *et al.,* 2005; Omemu and Aderoju, 2008). other studies have also shown that microorganisms improper storage at household level before cooking can encourage growth of microorganisms some of which may be pathogenic (Omemu *et al.,* 2007b). Cooking of food not only

improves the taste, smell, appearance and digestibility, it also reduces the number of microorganisms, improves keeping qualities by inhibiting moulds, yeast and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving the overall safety of food. Where food is allowed to stand in high ambient temperatures after being prepared (due to lack of refrigeration facilities, fuel and mother's time needed to reheat foods), considerable multiplication of pathogenic bacteria may occur, increasing the risk of diarrhoea in the young child still further (Black *et al.,* 1989). This situation is particularly critical when foods are consumed without reheating and when reheating temperatures are typically well below levels capable of destroying pathogens.

4.1. Enumeration of Isolates

In the present study, we have isolated both indicator and pathogenic microorganisms and unfortunately neither of the food samples collected could meet the microbiological standard in terms of total viable count (TVC) or total Staphylococci count (TSC). This is also similar to the findings of Rabbi et al. (2011) in Bangladesh. Although there is no available epidemiological data about the risks of food-borne diseases resulting from these bole fish supply in Nigeria, sparse information about the risk of street-vended foods in other developing countries has been published (Rabbi et al., 2011).

4.2. Total viable count (TVC)

According to Adams and Moses (2008), the normal bacterial load of the surface slime of fish can range from 10^2 – 10^7 cfu/cm2 and the Gills and Intestines can range up to 10^3 and 10^7 cfu/g respectively. In this study, the total viable counts of the street-vended ready-to-eat bole fishes sampled in this study ranged from $2.8x10^3$ to $6.3x10^5$ cfu/g. The bacterial load of the bole fish samples fell below the range of the maximum limit (10^6 cfu/g) recommended by the international commission for microbiological standards of foods (ICMSF, 1978; Okoro et al., 2010; Adebayo-Tayo et al., 2012d). Assessment of food safety knowledge and practices of abattoir and butchery shops in the Mekelle city, Ethiopia by Mekonnen et al. (2011) and Haileselassie et al. (2012) showed that meat samples collected from butchery shops had viable bacterial load in the range of $1.1x10^5$ to $4.3x10^6$ cfu/g.

4.3. Total Staphylococci count (TSC)

The total Staphylococci count (TSC) for street-vended roasted bole fishes ranged from $1.6x10^3$ to $2.3x10^5$cfu/g and $2.3x10^3$ to $1.9x10^5$ cfu/g for fresh Atlantic horse mackerel.

4.4. Total coliform count (TCC)

Similar to results of total viable count, ready-to-eat bole fish sold in the streets of Port Harcourt had the highest number of total coliforms counts (1560 MPN/g) with an average of 1100 MPN/g at the second sampling of the vending day. Alyaaqoubi et al. (2009) reported a similar value on beef curry (1100 MNP/g with an average of 625 MPN/g). Even so, total coliform counts obtained from aseptically prepared bole fish in this study was still lower compared to tsire-suya, a traditional RTE meat in Nigeria, which had coliform growth varying from $1x10^2$ to 4.2×10^3 cfu/g (Uzeh et al. 2006). It was expected that both Fresh Atlantic Horse Mackerel and street vended bole fish would incur heavy growth of coliforms and other microorganisms, because these positions were open to different sources of contamination such as cars exhausts, rising dusts and garbage which provide a hiding place for insects and animal pests (Bryan et al. 1997; Alyaaqoubi et al., 2009). In case of the total coliform counts, Rabbi et al. (2011) also reported the highest occurrence in fish (1.6×10^7 cfu/g) and egg (2×10^6 cfu/g) curry samples. Arannilewa et al. (2006) also found that the total coliform count range in fish was between 3.0×10^3 - 7.5×10^6 with increasing values, as the duration of storage increases. The presence of total coliforms led us to assume the presence of other harmful and pathogenic microorganisms such as *Salmonella* spp (Rabbi et al., 2011).

4.5. Isolation and Identification of Isolates

In this study, two gram positive and two gram negative bacteria were isolated; *Staphylococcus aureus* (35.7%), *E. coli* (17.9%), *Klebsiella sp* (21.4%), and *Bacillus sp* (25.0%) while fungal isolates were *Neurospora* sp (16.7%), *Aspergillus flavus* (25.0%), *Aspergillus niger* (41.7%) and *Penicillium* sp (16.7%). This is consistent with the findings of previous studies in Nigeria and outside Nigeria. Studies conducted in different parts of Ethiopia showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like campylobacter, *Salmonella, Staphylococcus aureus, Bacillus cereus and Escherichia coli* (Kinfe and Abera, 2007; Tefera et al., 2009; Mekonnen et al., 2011; Haileselassie et al., 2012).

4.6. *Staphylococcus sp.*

Staphylococcus sp was most predominant (35.7%) bacterial isolates in this study. Coagulase-positive Staphylococci were found in all the examined samples. This deviated from the findings of

other previous studies (Alyaaqoubi et al., 2009). Food poisoning by *Staphylococcus* affects hundreds of thousands of people each year (Rabbi et al., 2011). *Staphylococcus sp.* produces toxins that withstand high temperatures and are spore forming which germinate and release enterotoxins. Ingestion of toxin- containing food causes nausea, vomiting, abdominal cramps and diarrhea. In most studies, *S. aureus* are amongst the most common pathogens found on hands (Shojoei et al., 2006; Rabbi et al., 2011). It is also revealed that most street-vended food handlers were carriers of *S. aureus* (Rabbi et al., 2011).

In a study by Alyaaqoubi et al. (2009), coagulase-positive Staphylococci were not found in all the examined samples. Similarly, Hanashiro et al. (2005) stated that Staphylococci were not detected in home- and street-prepared RTE meal samples in Sao Paulo, Brazil. On other hand, all samples failed to meet the standards set for *E. coli* and coagulase-positive Staphylococci. This also deviated from the findings reported by Alyaaqoubi et al. (2009). A study by Fang et al. (2003), 10.0% and 12.0% of the RTE meat products were observed to exceed the Chinese government standard for *E. coli* and *S. aureus*, respectively. The percentages of non-compliant samples in this study suggest that the ready-to-eat bole fishes sampled in the Port Harcourt were nor of any high microbiological quality, consequently as they were not properly prepared, stored and handled.

4.7. *Bacillus sp.*

Bacillus sp (25.0%) was second predominant bacterial isolates in this study. *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). Since some species of *Bacillus* are airborne and dust- borne contaminant, exposing of the roasted fish and poor handling can lead to contamination.

4.8. *Klebsiella* spp.

This is the third predominant (21.4%) in this study. The presence of *Klebsiella* spp. on street-vended ready-to-eat bole fishes sold by vendors further confirms the role of cross contamination by the bole fishes. This microorganism possesses risk to public health. 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). *Klebsiella* species exist as normal flora in the gastrointestinal tract of animals and humans (Siri et al., 2011). 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). *Klebsiella* sp attacks the respiratory tracks of an individual. This colonization leads to the chronic lung infections with which the species is associated. This infection is promoted by the large capsules, which prevent phagolytosis and some strains of *Klebsiella* produce toxins. Furthermore, similar studies were carried out in Malaysia to demonstrate the presence of specific microorganisms in certain foods such as prevalence of Salmonella in raw and cooked foods in Malaysia (Alyaaqoubi et al., 2009) and incident of *Klebsiella pneumonia*e in street foods sold in Malaysia (Haryani et al. 2007; Alyaaqoubi et al., 2009).

Domestic animals such as cattle and horses are principal hosts for *Klebsiella* species (Siri et al., 2011). Improper farm management techniques and/or improper hygiene may facilitate contamination of water sources with *Klebsiella* species (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). *Klebsiella* species have been found to cause infections in babies through the consumption of powdered infant formula that was contaminated with pathogenic strains (Liu et al., 2008; Siri et al., 2011). Improper deposition of human faeces can lead to contamination of the soil with *Klebsiella* species hence *Klebsiella pneumonia* has been isolated from vegetables such as dried bush okra (*Corchorus olitorius*) and African spider herb (*Cleome gynandra*) (Siri et al., 2011).

4.9. *Escherichia coli*

E. coli was least predominant (17.9%) bacterial isolates in this study. The presence of *Escherichia coli* on street-vended ready-to-eat bole fishes sold by vendors also further confirms the role of cross contamination by the bole fishes. The isolation of *Escherichia coli* and *Klebsiella* spp. from the samples of bole fishes poses food safety problems since they are all enterotoxigenic and cause gastroenteritis (Adu-Gyamfi and Nketsia-Tabiri, 2007). *E. coli* because of its adhesion properties; that is, its well-developed K. antigen capsule makes it very easy to stick on the sample (roasted fish) especially when it is exposed mostly in busy areas, which can cause diarrhea and also infertile. Though

Escherichia coli was least predominant in this study, it not found in a food sold at cafeteria in a study by Alyaaqoubi et al. (2009). According to Alyaaqoubi et al. (2009), neither fried chicken nor chicken curry from all sampling sites contained *E. coli* at the beginning of vending day. 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.

4.10. *Salmonella* spp.

In our study, *Salmonella* spp count was nil. 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 also identified pathogens including *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).

This study is in agreement with the studies reported by Soriano et al. (2001) who found no *Salmonella* in raw and RTE samples from 20 University restaurants in Valencia, Spain and Alyaaqoubi et al. (2009) who also found no Salmonella in some selected ready-to-eat food at Hulu Langat district, Malaysia. Umoh and Odoba (1999) also indicated that none of the RTE food samples from mobile food sellers were contaminated with *Salmonella*, similar with results of study done by Aycicek et al. (2004) in a military hospital in Ankara, Turkey. Meldrum et al. (2006) from the United Kingdom also revealed the absence of this pathogen during the investigation of the microbiological quality of RTE foods between 2003 and 2005 in Wales. The absence of *Salmonella* in these studies indicated that good handling practices during the cooking process and good storage facilities were available for both raw and RTE meat and poultry items used in the meals (Alyaaqoubi et al., 2009).

4.11. *Aspergillus flavus* and *Aspergillus niger*

Aspergillus niger (41.7%) is the most predominant while *Aspergillus flavus* (25.0%) was the second predominant fungal isolates in this study. 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. The presence of *Aspergillus* in this study reveals possible production of aflatoxins (Adebayo-Tayo et al., 2006, 2012a,b). However, contrary to the findings of this present study, Adebayo-Tayo et al. (2012b) reported that *Mucor* spp. and *Rhizopus* spp. (37.5%) were the most predominant fungal isolates, followed by *Penicillum* spp. (18.8%). *Aspergillus spp*. was the least prevalent (6.3%) in their study on the microbiological and physicochemical level of fresh catfish (*Arius hendelotic)* from different markets in Akwa Ibom State, Nigeria. In another study by Adebayo-Tayo et al. (2012b), *Aspergillus niger* was the least prevalent (25.0%).

4.12. *Penicillium* sp. and *Neurospora* sp.

In this study, *Penicillium* sp. (16.7%) and *Neurospora* sp. (16.7%) were least predominant. 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. Generally, the mould observed is pathogenic and cause harm to humans (Adebayo-Tayo et al., 2012c). *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions (Doyle, 2007; Adebayo-Tayo et al., 2012c). Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) (Doyle, 2007; Adebayo-Tayo et al., 2012c).

However, 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). 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).

The high rate of microbial contamination of fresh fish could be explained by the practice of scale and evisceration (Roberto *et al*., 2006; Akaki et al., 2012) exclusively manual using contaminated equipment and work surfaces added to it poor clean water (Oranusi *et al*., 2007; Akaki et al., 2012). Our study was especially designated the equipment and utensils used, the absence of cold throughout the process, the work (healthy carrier) and the behavior of the manipulators (not maintained hair, uncut nails, wearing rings or watches, manipulation of money, unsuitable dress) as the possible factors responsible for the presence of these microorganisms found in the fish samples (Bukar *et al*., 2010; Akaki et al., 2012).

Our results are in agreement with the conclusions of the study of Barro *et al*. (2006) and Akaki et al. (2012). They were underline deficient practices on the food hygiene sold in the streets of Port Harcourt, Nigeria mainly because of the lack of understanding of the potential sources of contamination by the meal producers. Cooking and roasting have beneficial effect if we consider the significant reduction in the proportion of contaminated samples. The beneficial effect of heat treatment on the reduction of the levels of contamination is recognized in the food process (Akaki *et al*., 2008; Akaki et al., 2012). However, the manual manipulation during cooking, the poor maintenance of utensils, insalubrious working environment are sources of re-contamination of ready-to-eat bole fish (Barro *et al*., 2006; Oranusi *et al*., 2007; Akaki et al., 2012).

The above mentioned results were not consistent with those obtained from the study conducted by Aycicek et al. (2004) who evaluated total coliforms and *E. coli* according to the Turkish Standard Institution and Alyaaqoubi et al. (2009) who evaluated microbiological quality of selected ready-to-eat food at Hulu Langat district, Malaysia. The critical control points for bole fish used in this study are purchasing raw fish from street vendors, inadequate cooking, and addition of ingredients after heat application and prolonged holding at ambient temperature. Possibly, cross-contamination occurred to the foods between these intervals. The women vendors lack good personal hygiene, which is vital in reducing the chance of contamination of foods (Amusan et al., 2010). It is not surprising in such poor hygiene and handling situation to isolate *Staphylococcus* spp from the majority of samples. So there is every possibility of contamination before or after cooking of a food as well as during serving (Rabbi et al., 2011). Possible sources of contamination may account from washing water, insects and rodents, contaminated hands or people having skin infection, hair or hair products in food, unhygienic kitchen environment, contaminated equipment, contaminated air or dust, personal hygiene, lack of adequate sanitation etc (Rabbi et al., 2011).

5. CONCLUSION

The microbiological quality of the street-vended ready-to-eat bole fishes in this study has revealed that many factors contributed to the contamination of bole fishes. However, based on the acceptable level of the microbiological guideline/standards, the total viable count, Staphylococcus count, and total coliform counts were exceeded in all samples collected at intervals of marketing times. Therefore, this study has shown that despite the heating of the fish, microorganism was still observed. It showed that good personal hygiene when handling foods can go a long way to minimizing contamination. Some recommendations on how to minimize risk in public health in consumption of roasted fish include; that laws and regulations should be put in place in order to check the standards of roasted fish and other ready to eat food. The fish should be stored in a warm air-tight container like 'show-glass' with bulb to provide constant heat. Individuals preparing roasted fish should be educated on the pathogenic microorganisms that can be introduced if they do not practice a good personal hygiene and proper storage. Subsequent study should focus on roasted fish wrapped with newspaper because the ink can be a source of contamination. Therefore, if all recommendations are put into consideration then, it will be safe to consume roasted fish.

REFERENCES

1. Adams MR, Moses MO. Food Microbiology (third edition). The Royal Society of Chemistry, Cambridge, UK. 2008, 179 pp.
2. Adebayo-Tayo BC, Adegoke AA and Akinjogunla OJ. 2009. Microbial and physico-chemical quality of powdered soymilk samples in Akwa Ibom, South Southern Nigeria. African Journal of Biotechnology, 8 (13): pp. 3066-3071
3. Adebayo-Tayo BC, Odu NN, Esen CU, Okonko IO. Microorganisms Associated With Spoilage Of Stored Vegetables In Uyo Metropolis, Akwa Ibom State, Nigeria. Nature and Science 2012c;10(3):23-32
4. Adebayo-Tayo BC, Odu NN, Igiwiloh NJPN, Okonko IO. Microbiological and Physicochemical Level of Fresh Catfish (*Arius*

hendelotic) From Different Markets in Akwa Ibom State, Nigeria. New York Science Journal 2012b;5(4):46-52
5. Adebayo-Tayo BC, Odu NN, Okonko IO. Microbiological and physiochemical changes and its correlation with quality indices of tilapia fish (*Oreochromis niloticus*) sold in Itu and Uyo markets in Akwa Ibom State, Nigeria. New York Science Journal 2012d;5(4):38-45
6. Adebayo-Tayo BC, Onllude AA, Ogunjobi AA. 2006. Bacteriological and Proximate Analysis of Periwinkle from two different creeks in Nigeria. *World Applied Science Journal 1* (2): 87-91.
7. Adebayo-Tayo BC; Odu NN; Anyamele LM; Igwiloh NJPN; Okonko IO. Microbial Quality Of Frozen Fish Sold In Uyo Metropolis. Nature and Science 2012a; 10(3): 71-77
8. Adu-Gyamfi A. and Nketsia-Tabiri J. 2007. Microbiological Studies of Macaroni and Vegetable Salads in *Waakye*, a Local Street Food. *Ghana J. Sci*. 47:3-9
9. Akaki KD, Sadat AW and Coulibaly K. 2012. Bacteriological Study of Grilled Fish Consumed in Yamoussoukro Commune, Political Capital of Côte d'Ivoire. Current Research Journal of Biological Sciences 4(3): 297-300
10. Akaki, K.D., A.W.S. Loiseau and G.J.P. et Guyot, 2008. Study of the behavior of strains of *Bacillus cereus* ATCC 9139 and *Escherichia coli* ATCC 25922 by the method of challenge-tests when making porridge made from fermented millet paste from Ouagadougou (Burkina Faso). Rev. Ivoir. Sci. Technol., 11: 103-117.
11. Akintobi AO, Okonko IO, Akano OR, Agubiade SO, Onianwa O. Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. Academia Arena 3(11): 1-10
12. Al-Hindi RR, Al-Najada AR, Mohamed SA. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. African Journal of Microbiology Research, 2011; 5(4): 443-448.
13. Alyaaqoubi SJM, Sani NA, Abdullah A, and Abdul Rahman RD. 2009. Microbiological Quality Of Selected Ready-To-Eat Food At Hulu Langat District, Malaysia. *Prosiding Seminar Kimia Bersama Ukm-Itb Viii 9-11 Jun 2009; 421-433*
14. Amusan E, Oramadike C.E, Abraham-Olukayode A.O, and Adejonwo O.A. 2010. Bacteriological Quality of Street Vended Smoked Blue Whiting (*Micromesistus poutasou*). Internet Journal of Food Safety, 12:122-126
15. Arambolu, P., Almeida~ C.R., Cuelar, J., Belotto, A.J. (1993). Some street food vending .in Latin America: some insights into its socio-cultural imperatives and public. health implications. Proceedings of 11th International Symposium of World Assoc. Vet. Food Iiygienists (WAVFH), October, 24-29,1993, Bangkok, Thailand,pp. 405-425.
16. Aycicek, H., Sarimehmetoglu, B. and Cakiroglu, S. 2004. Assessment of the microbiological quality of meals sampled at the meal serving units of a military hospital in Ankara, Turkey. *Food Control* 15: 37-384.
17. Barro, N., A.R. Bello, A. Savadogo, C.A.T. Ouattara, A.J. Ilboudo and A.S. Traoré, 2006. Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). Afri. J. Biotechnol., 5(11): 1107-1112.
18. Black, R.E., Lopez de Romana, K.H.G., Brrown, N. B., Bazalar, O.G. and Kanashiro, H.C. 1989. The incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. American Journal of Epidemiology 189: 785-799.
19. Bryan, F.L., Jermini, M., Schmitt, R., Chilufya, E.N., Mwanza, M., Matoba, A., Mfume, E. and Chibiya, B. 1997. Hazards associated with holding and reheating foods at vending sites in a small town in Zambia. Journal of Food Protection 60: p391-398
20. Bryan, M.B., V. Caroline and F. Madelon, (2003). Safety of vendor-prepared foods: Evaluation of 10 processing mobile food vendors in Manhattan, Public Health Reports 118, pp. 470-476.
21. Bukar, A., A. Uba and T.I. Oyeyi, 2010. Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria. Afri. J. Food Sci., 4(2): 032-036.
22. Canet, C. and C. N'Diaye, (1996). Street foods in Africa, Food Nutrition and Agriculture 17/18, pp. 4-13.
23. Chukwuka, KS, Okonko, IO, Adekunle AA. Microbial Ecology Of Organisms Causing Pawpaw (Carica Papaya L.) Fruit Decay in Oyo State, Nigeria. American-Eurasian Journal of Toxicological Sciences, 2010; 2 (1): 43-50
24. Doyle EM. 2007. FRI BRIEFINGS: Microbial Food Spoilage: Losses and Control Strategies. *A Brief Review of the Literature*. Food Research Institute, University of Wisconsin–Madison.

http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf
25. Elmahmood, A.M. and J.H. Doughari, 2007. Microbial quality assessment of kunun-zaki beverage sold in Girei Town of Adamawa State, Nigeria. Afri. J. Food Sci., 1(1): 011-015.
26. Esrey, S.A. and Feachem. R.G. 1989. Interventions for the control of diarrhoeal diseases among young children: promotion of food hygiene. Geneva: Control of Diarrhoea disease programme. WHO, 1-22. (WHO/CDD/89, Research Report. No. 30).
27. Fang TJ, Chen CY, Kuo WY (1999). Microbiological quality and incidence of *Staphylococcus aureus* and *Bacillus cereus* in vegetarian food products. Food Microbiol., 16: 385-391.
28. Fang, T. J., Wei, Q. K., Liao, C. W., Hung, M. J. & Wang, T. H. 2003. Microbiological quality of 18 oC ready-to-eat food products sold in Taiwan. *International Journal of Food Microbiology* 80: 241– 250.
29. Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed. (rev. A). AOAC International, Gaithersburg, Md.
30. Gadaga, T. H., Samende B. K., Musna C. & Chibanda, D. 2007. The microbiological quality of informally vended foods in Harare, Zimbabwe. *Food control* 20: 149-156. www.elsevier.com/locate/foodcont. html [12/10/2007.
31. Gibbons, S., Adesiyun, A., Seepersadsingh, N. & Rahaman, S. 2006. Investigation for possible sources of contamination of ready-to-eat meat products with *Listeria* spp. and other pathogens in a meat processing plant in Trinidad. *Food Microbiology* 23: 359-366.
32. Haileselassie M, Taddele H and Adhana K. 2012. Source(s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks in Mekelle City, Ethiopia. ISABB Journal of Food and Agriculture Science, 2012; 2(2): 20-29
33. Hanashiro, A., Morita, M., Matte, G.R., Matte, M.H. & Torres, E.A.F.S. 2005. Microbiological quality of selected street foods from a restricted area of Sâo Paulo city, Brazil. *Food Control* 16: 439-444.
34. Haryani Y, Noorzaleha AS, Fatimah AB, Noorjahan BA, Patrick GB, Shamsinar AT, Laila RAS, Son R (2007). Incidence of *Klebsiella pneumonia* in street food sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD-PCR analysis. Food Cont., 18: 847-853.
35. Ifediora, A.C., Nkere, C.K. and C.U. Iroegbu. 2006. Weaning food preparations consumed in Umuahia, Nigeria: Evaluation of the Bacteriological quality. Journal of Food Technology 4(2): 101-105.
36. Knife Z, Abera K (2007). Sanitary conditions of food establishments in Mekelle town, Tigray, north Ethiopia. Ethiopian J. Health Dev., 21(1): 3-11.
37. Lau HY, Clegg S, Moore TA (2007). Identification of *K. pneumonia* genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. Microb. Pathog., 42: 148-155.
38. Liu Y, Liu C, Zheng W, Zhang X, Yu J, Gao O, Hou Y**,** Huang X (2008). PCR detection of *Klebsiella pneumoniae* in infant formula based on 16S–23S internal transcribed spacer. Inter. J. Food Microbiol., 125(3): 230-235.
39. Mekonnen H, Habtamu T, Kelali A, Shewit K (2011). Study on food safety knowledge and practices of abattoir and butchery shops in Mekelle City, Ethiopia (un published).
40. Meldrum, R. J., Smith, R. M. M., Ellis, P. & Garside, J. 2006. Microbiological quality of randomly selected ready-to-eat foods sampled between 2003 and 2005 in Wales, UK. *International Journal of Food Microbiology* 108: 397-400.
41. Mensah P, Yeboah-Manu D, Owusu- Darko K, Ablordey (2002). A Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organization. Int. J. Public Health. WHO, Geneva, 80(7): 546-554.
42. Notermans, S.; G. Gallhoff, M. Zweitering and G., Mead (1995). Identification of critical control points in the HACCP system with a quantitative effect on the safety of food products, Food Microbiology 12, pp. 93-98.
43. Obayelu, A.E., V.O. Okoruwa and O.A. Oni, 2009. Analysis of rural and urban households' food consumption differential in the North-Central, Nigeria: A micro-econometric approach. J. Develop. Agric. Econ., 1(2): 018-026.
44. Omemu A.M and Omeike, S.O. (2010) Microbiological hazard and critical control points identification during household preparation of cooked *ogi* used as weaning food. *International Food Research Journal 17:* 257-266.
45. Omemu, A. M., Edema, M. O. and Bankole, M. O. 2005. Bacteriological assessment of street vended ready to eat (RTE) vegetables and pre-packed salad in Nigeria. Nigerian Journal of Microbiology 19(1–2): 497–504.

46. Omemu, A.M. and Aderoju, S.T. 2008. Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. Food Control 19: 396–402.
47. Omemu, A.M., Bankole, M.O., Oyewole, O.B. and Akintokun, A.K. 2007b. Yeasts and moulds associated with *ogi* - a cereal based weaning food during storage. Research Journal of Microbiology 2 (2): 141-148.
48. Omemu, A.M., Oyewole, O.B. and Bankole, M.O. 2007a. Significance of yeasts in the fermentation of maize for *ogi* production. Food Microbiology 24: 571–576.
49. Oranusi S, Galadima M, Umoh VJ and Nwanze PI. 2007. Food safety evaluation in boarding schools in Zaria, Nigeria, using the HACCP system. Scientific Research and Essays 2 (10): 426-433
50. Rabbi FA, Rabbi F, Runun TA, Zaman K, Rahman MM and Noor R. 2011. Microbiological Quality Assessment of Foods collected from Different Hospitals within Dhaka City. Stamford Journal of Microbiology, 1(1):31-36
51. Redmond E.C. and and C.J. Griffith, 2003 Consumer food handling in the home: A review of food safety studies, Journal of Food Protection, 66 (1) pp. 130-161.
52. Roberto, G.C., N.M. dos Santos, A.N. de Medieros, R. de Cassia Ramos Queroga and M.S. Madruga, 2006. Microbiological evaluation of precooked goat "BUCHADA". Brazil. J. Microbiol., 37: 362-367.
53. Shojoei, H., J. Shooshtaripoor, and M. Amiri. 2006. Efficacy of Simple Hand-Washing in Reduction of Microbial Hand Contamination of Iranian Food Handlers. Food Research International, 2006. 39:525 –529.
54. Siri GP, Sithebe NP and Ateba CN. 2011. Identification of *Klebsiella* species isolated from Modimola dam (Mafikeng) North West Province – South Africa. African Journal of Microbiology Research, 5(23):3958-3963
55. Soriano, J. M., Rico, H., Molto, J. C. and Manes, J. 2001 Incidence of microbial flora in lettuce, meat and Spanish potato omelette from restaurants. *Food Microbiology* 18: 159-163.
56. Speck, M.L. 1976. Compendium of Methods for Microbiological Examination of Foods. American Public Health Association, Washington DC, pp. 277–328.
57. Tefera W, Daniel A, Girma Z (2009). Prevalence of Thermophilic Campylobacter species in carcasses from sheep and goats in an abattoir in Debre Zeit area, Ethiopia. Ethiopian J. Health Dev., 23(3): 230
58. Umoh, V. J. and Odoba, M. B. 1999. Safety and quality evaluation of street foods sold in Zaria, Nigeria. Food Control 10: 9–14.
59. Umoh, V.J., Adesiyun, A.A., and Gomwalk, N.E. 1999. Assay for *Staphylococcus aureus* growth and enterotoxin in three fermented milk products. Zariya Veterinary 6: 7–15.
60. Uzeh, R. E., Ohenhen1, R. E. & Adeniji, O. O. 2006. Bacterial Contamination of *Tsire-Suya*, a Nigerian Meat Product. *Pakistan Journal of Nutrition* 5 (5): 458-460. www.elsevier.com/locate/jfca.html [28.10.2007]
61. World Health Organization (1996). Essential safety requirements for street vended foods. Food Safety Unit, Division of Food and Nutrition, WHOIFNUIFOSf96.7.
62. World Health Organization (2000). Food safety: Resolution of the Executive Board of the WHO. 105th session.
63. Yusuf, I.Z., Umoh, V.J. and Ahmad, A.A. 1992. Occurrence and survival of enterotoxigenic *Bacillus cereus* in some Nigerian flour-based foods. Food Control 3:149–152.

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