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MICROBIAL ASSESSMENT OF FROZEN FOODS SOLD IN AYOBO, LAGOS

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ABSTRACT

This study seeks to investigate the microbial profile of frozen fish and meat. Forty samples consisting of *Scomber scombrus* **(**Titus), *Clupea harengus* (Shawa) and frozen meat (Chicken, Turkey) were purchased from different retail outlets in Ayobo-Ipaja markets for microbiological analysis. The samples were analysed for the total viable count using standard microbiological procedures. The mean bacterial and fungal counts for *Scomber scombrus*, Chicken, *Clupea harengus* and Turkey are 254.70±83.81 CFU/G and 5.50±4.45 CFU/G; 210.10±55.03 CFU/G and 6.80±3.39 CFU/G; 298.20±67.35 CFU/G and 6.10±3.87 CFU/G; 221.30±80.33 CFU/G and 4.30±2.00 CFU/G respectively. *Clupea harengus* has the highest bacterial count while *Scomber scombrus* has the lowest bacterial count. Chicken has the highest fungal count while Turkey had the lowest fungal count. The microbial isolates from the frozen food samples include species of *S. aureus*, *E. coli*, *Salmonella*, *Micrococcus*, *Aspergillus*, and *Penicillium*. *Escherichia coli* were susceptible to all the antibiotics while *Salmonella* sp., *Staphylococcus aureus*, and *Micrococcus* were resistance to Augmentin, Gentamycin, Tarivid, and susceptible to Sparfloxacin and Chloramphenicol. Although freezing retard pathogens multiplication, post-harvest contaminants can multiply during thawing to a level that can have a major impact on the quality of the final consumer product. It is advised that frozen foods must be properly cooked before consumption and effective hazard analysis and critical control point implemented.

Keywords: Antibiotic resistance, *Scomber scombrus*, *Clupea harengus* and frozen meat

INTRODUCTION

Frozen foods are foods that are been refrigerated so they can be protected to remain for a significant period. These includes frozen meats (chicken, turkey, gizzard), frozen fish, frozen yoghurt, among numerous others. Freezing of these foods preserves them for longer periods by halting the development and multiplication of microorganisms that cause both food waste and foodborne ailment and by upsetting the food source's protein action (**Oranusi *et al*., 2014**). Frozen foods do not need any additional additives since microorganisms do not develop when the temperature of the food is beneath −9.5 °C (15 °F), which is adequate in forestalling food deterioration. Most microbes do not increase at frigid temperature and a large number of these microorganisms die because their enzymes do not work appropriately to keep up ordinary cell activities (**Baek *et al*., 2009**).

Freezing is the prevailing method of preserving high quality in foods. It inhibits the growth of microbes by reducing their numbers but not destroying them. The pathogen that survives the freezing process constitutes a threat to the consumers. Survival depends on the category of the microbes, freezing range, freezing procedures and thawing, the type of food and the substrate composition (**Baek *et al*., 2009**). Some of the pathogens present in frozen include *Salmonella*, *Staphylococcus spp*., *Escherichia spp*., *Vibrio parahemolyticus*, *Clostridium perfringens*, *Clostridium botulinum*, *Enteroviruses*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Salmonella enteric serovar typhimurium* (**Ismail & Belma, 2002**). Presence of these pathogenic microbes could cause foodborne disease such as cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, e.t.*c*. (**Ismail & Belma, 2002**).

The microbiological safety of frozen food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (**Adebayo-Tayo *et al.*, 2012**). The Hazard Analysis Critical Control Point (HACCP) concept is used to identify microbiological vulnerable points in the food production process and processing, to determine the most appropriate methods of control to be applied, usually such methods as improved handling techniques, monitoring of temperature and more intensive supervision (Okonko *et al.*, 2008). Quality and safety of frozen food are the aspects affecting the overall consumer acceptability in terms of flavour, texture, aroma, colour and appearance besides microbiological safety and nutritional quality (**Nyenje &Ndip, 2013**). Microbial food safety is an increasing public health concern worldwide. It is estimated that food contamination with pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing and handling or preparation (Elhadi, 2014). Consumers are also a link in the chain of food-borne human diseases, because of the way they store and cook meat, poultry meat, fish and their products (**Bhaisare *et al.*, 2014**).

Food is an excellent means of introducing pathogenic microorganisms to the general population and in immuno-compromised people, and therefore it may transfer antibiotic-resistant bacteria to the intestinal tract of consumers very efficiently (**Baek *et al.*, 2009**). Drug resistance is spreading fast mainly due to overuse of antibiotics, incomplete and underuse of medications and widespread practice of feeding livestock low levels of antibiotics to promote growth (Elhadi, 2014). Due to the frequency of consumption of frozen foods within the Nigerian populace, there may be an opportunity of the extended hazard of contamination and change in the susceptibilities of the microorganisms that could be isolated from those merchandised and this could affect the health of the consumers. Hence, this study seeks to investigate the microbial load and the antimicrobial susceptibility of microorganisms isolated from frozen foods sold in Ayobo markets, a suburb in Alimosho local government area, Lagos State, South-western Nigeria.

MATERIALS AND METHOD

Study Location

Ayobo is a suburb and densely populated area in Alimosho local government area Lagos State, South-western Nigeria. Ayobo has the region font code of Africa/ Middle East, and it is located at an elevation of 42 meters above sea level with a population up to 71,657. Its coordinates are 6°36'0" N and 3°13'60" E in DMS (Degrees Minutes Seconds) or 6.6 and 3.23333 (in decimal degrees).

Sample Collection

Forty samples made up of ten each of; Titus- *Scomber scombrus*; Shawa - *Clupea harengus* and Poultry meat- Chicken and Turkey were purchased from different sales outlets (cold rooms and retail stores) in Ayobo, Lagos state during October 2020. These purchased samples were placed in sterile bags and transported in a cold pack to the laboratory for analysis within one hour of collection.

Preparation of Media and Samples

Ten grams of every frozen food samples were weighed and placed into 10 ml of 1% peptone water and blended for (30–60) seconds in a sterilized blender machine. Rigorous shaking was done with the use of a mechanical shaker after which, a serial dilution of the homogenate was carried out in sterile universal bottles using 9ml of 0.1% sterile peptone water up to 10^{-10} dilution. This was done under aseptic condition. The culture media used for microbiological analyses include nutrient agar, Macconkey agar, potato dextrose agar, eosin methylene blue agar and mannitol salt agar, each were prepared according to manufacturers' instruction.

Isolation of Microorganisms

One millilitre from the dilutions were inoculated on Nutrient agar, MacConkey agar, potato dextrose agar for the enumeration of total microorganism, coliform count and enumeration of fungi respectively. After inoculation, Petri dishes containing Nutrient agar, MacConkey agar, Eosin methylene blue and Mannitol salt agar were incubated at 37ºC for 24 h, while inoculated plates containing Potato dextrose agar was incubated at 28ºC for 3-7 days.

Identification and Characterization of Bacterial isolates

The isolated cultures were characterized and identified based on their cultural, morphological, and biochemical tests.

Antibiotic susceptibility test

Mueller-Hinton agar was prepared according to the manufacturer's instructions. Three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The growth was transferred into a tube containing 4-5 ml of nutrient broth. The nutrient broth was incubated until it achieved the turbidity of the McFarland standard solution. The swab which contains selected isolated colonies was dipped into the inoculums suspension and then streaked on the dried surface of the Mueller-Hinton agar plate (Merck, Germany), according to CLSI (**CLSI, 2018**). The surface was streaked two more times rotating the plate approximately 60˚ each time to ensure an even distribution of the inoculums. The antibiotic discs used were: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxacillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) for Gram negative isolates. while SXT; Septrin (30µg), CH; Chloranphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg) for Gram positive isolates. Each disc was pressed down to ensure complete contact with the agar surface. The plates were then inverted and placed in the incubator at 35ºC (**Lalitha, 2004**). After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant were made according to **CLSI, (2018**) manual.

RESULTS

Table I Bacteria and Fungi Counts obtained from Frozen food samples

Sample		Mean± Std. Deviation (10^8) CFU/G
Turkey	BC	221.30±80.33
	FC	4.30±2.00
Titus (*Scomber scombrus)*	BC	254.70±83.81
	FC	5.50±4.45
Chicken	BC	210.10±55.03
	FC	6.80±3.39
Shawa (*Clupea haengus)*	BC	298.20±67.35
	FC	6.10±3.87

Key: BC- Bacterial count; FC- Fungal count

Table II ANOVA of the difference in the bacterial counts in frozen fish and meat product sampled

	Mean (10^8) CFU/G	Df	F	Sig (ρ)
Turkey	221.30±80.33	3, 36	2.98	0.044
Titus (*Scomber scombrus)*	254.70±83.81			
Chicken	210.10±55.03			
Sharwa (*Clupea haengus)*	298.20±67.35			

Table II shows that there is a significant difference in the bacterial counts obtained from the frozen fish and meat samples sold around Ayobo market (F (3, 39) = 2.98; $\rho < 0.05$). Using the LSD to determine where the significant difference is, the result obtained showed that there was a significant difference in the bacterial count between turkey and *Clupea harengus* (mean difference = -76.69*10^8 CFU/G, and between chicken and *Clupea harengus* (-88.10* 10^8) CFU/G.

Table III ANOVA of the difference in the fungal counts in frozen fish and meat product sampled

	Mean CFU/G	Df	F	Sig (ρ).
1 Turkey	4.30±2.00	3, 36	0.892	0.455
2 Titus (*Scomber scombrus)*	5.50±4.45			
3 chicken	6.80±3.39			
4 sharwa(*Clupea haengus)*	6.10±3.87			

Table III shows that there was no significant difference in the fungal count obtained from the frozen fish and meat sold in Ayobo (F (3, 39) = 0.892; $\rho > 0.05$).

Table IV Total viable count, Total and Faecal coliform counts in frozen fish and meat products

Samples	Dilution factor	TVC (CFU/g)	TC (CFU/g)	FC (CFU/g)
TURKEY	10^{-5}	300	256	165
	10^{-8}	268	220	142
CHICKEN	10^{-5}	256	217	189
	10^{-8}	216	205	124
TITUS (*Scomber scombrus)*	10^{-5}	392	286	206
	10^{-8}	272	216	195
SHARWA (*Clupea haengus)*	10^{-5}	387	284	288
	10^{-8}	356	215	266

KEY: TVC- Total viable count; TC- Total coliform; FC- Faecal coliform

Table V Microorganisms isolated from frozen fish and meats

SAMPLES	MICROORGANISMS ISOLATED
TURKEY	*Salmonella spp, Staphylococcus aureus Escherichia coli, Aspergillus spp*
SHAWA (*Clupea harengus)*	*Staphylococcus aureus, Micrococcus spp, Escherichia coli, Salmonella spp. Aspergillus spp.*
CHICKEN	*Staphylococcus aureus, Micrococcus spp, Escherichia coli, Penicillium spp., Aspergillus spp.*
TITUS (*Scomber scombrus)*	*Staphylococcus aureus, Micrococcus spp, Aspergillus spp., Escherichia coli.*

TABLE VI Identification of Organisms using biochemical tests

ISOLATE	A	B	C	D
Cell morphology	Cocci	Rods	Cocci	Rods
Colony morphology	Yellow, small, irregular	Mucoid	Light red small colonies	Large white and mucoid
Gram stain	+	-	+	-
Catalase test	+	+	+	+
Citrate test	+	+	+	-
Glucose	+	+	+	+
Lactose	-	+	+	-
H_2S Test	-	-	-	+
Coagulase test	+	-	-	+
Organisms identified	***Staphylococcus aureus***	***Escherichia coli***	***Micrococcus sp***	***Salmonella sp***

KEY: + = POSITIVE, - = NEGATIVE

Table VII Identification of Fungal Isolates

Cultural Characteristics	Morphological Characteristics	Fungi Identified
Whitish colony which turned from brown to black	From flasky surface to sporulation and septate hyphae	*Aspergillus* species
Green colony and profuse growth	From dark conidiophores and septate hyphae	*Penicillium* species

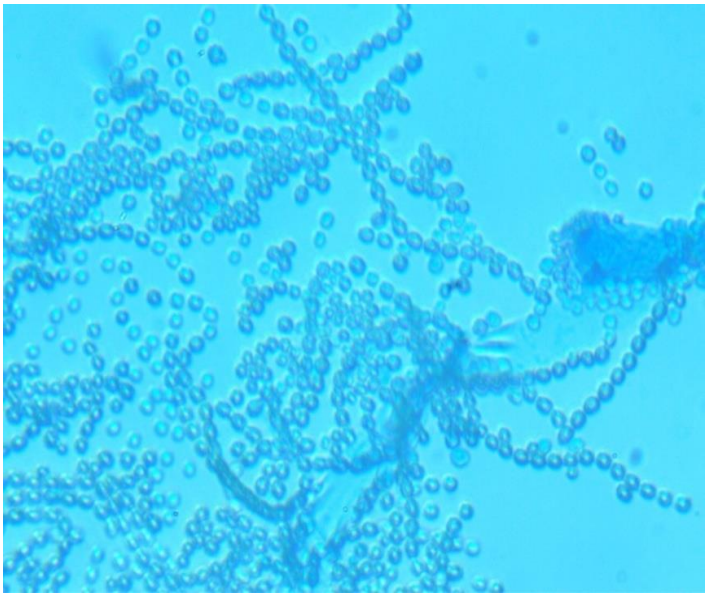

Figure I *Penicillium sp.* after staining with Lactophenol cotton blue showing spores in long chains.

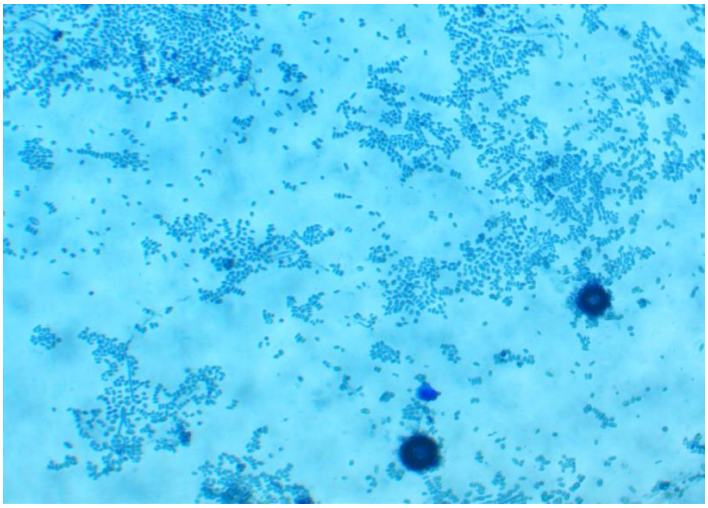

Figure II *Aspergillus sp* after staining with Lactophenol cotton blue.

Table VIII Antibiotic test result for Gram positive bacteria

Organism	AU	CN	PEF	AM	OFX	S	SXT	CH	SP	CPX
Staphylococcus aureus	R	R	S	S	R	R	R	S	S	R
Micrococcus sp.	R	R	S	S	R	R	R	S	S	R

Key: S –susceptible, R –resistance

Staphylococcus aureus, and Micrococcus were resistance to Augmentin, Gentamycin, Tarivid, and susceptible to Sparfloxacin and Chloramphenicol.

Table IX Antibiotic test result for Gram negative bacteria

Organism	SXT	CPX	AM	S	SP	CN	PEF
Salmonella sp.	R	R	R	R	R	S	S
Escherichia coli	S	S	S	S	S	S	S

Key: R; Resistance, S; Susceptible, +; Positive.

Escherichia coli were susceptible to all the antibiotics while *Salmonella sp.* was resistant to Septrin, Ciprofloxacin, Amoxacillin, and Streptomycin.

DISCUSSION

The results obtained from this study suggests that both meat and fish samples were contaminated by bacteria and fungi, irrespective of their frozen state and location. The microorganisms present in these samples include species of *Staphylococcus aureus, Micrococcus, Escherichia coli, Salmonella, Aspergillus* and *Penicillium.* The microorganisms isolated in this study are similar to what has been reportedly isolated in other studies in Nigeria (**Okonko et al., 2008; Kitai et al., 2005; Noor et al., 2013**). Fungal species of *Aspergillus* and *Penicillium* are spore bearers and common environmental contaminants of food and food products. The isolation of these organisms in fish and meat is in conjunction with the reports of **Zakki *et al.*, (2017); Ismail & Belma, (2002**). These fungi are observed as a pathogen in fresh and salt water fishes and have also been implicated in veterinary and human diseases (**Oranusi *et al.*, 2014**).

Staphylococcus aureus produces a variety of toxins, such as alpha toxins, beta toxins, enterotoxins, etc. These toxins can cause dehydration due to water loss during sweating, nausea, salivation and general weaknesses (**Kitai *et al.*, 2005**). *Escherichia coli* which thrives in the intestinal tracts of man is none pathogenic here but can harm man when it is displaced into the urinary tract (**Zakki *et al.*, 2017**). It is responsible for most nosocomial infections and neonatal meningitis. *Salmonella* causes salmonellosis and produces an endotoxin which inhibits phagocytosis. *Micrococcus sp.* are spoilage organisms whose presence indicates poor handling processes or techniques (**Okonko *et al.*, 2008**). These organisms have a host of other effects on man, but primarily, they are responsible for food infection and food intoxication.

The result showed that *E. coli* were susceptible to multiple antibiotics which were not comparable to what was reported by previous researchers **Lalitha, (2004); Nyenje & Ndip, (2013**). The *Salmonella* strains were resistant to gentamicin as comparable to findings in some other studies (**Kitai *et al.*, 2005; Noor *et al.*, 2013**)

Though epidemiological evidence outbreak of foodborne disease is scarce, there are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipment/utensils and the raw food materials (**Bhaisare et al., 2014**). Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of foodborne illness (**Adebayo-Tayo et al., 2012**). However, contamination of the fish may occur from food handlers and retailer who sell these items to the public for consumption (**Adebayo-Tayo et al., 2012; Zakki et al., 2017**).

CONCLUSION

The results from this study may be considered as additional knowledge to enhance proper controlling of the storage life of frozen foods quality in Ayobo. It also reveals that the frozen foods sold at the different markets in Ayobo community, Lagos state could be a source of food-borne bacterial and fungal pathogens. It has also shown that samples of frozen foods used in this study were highly contaminated by pathogenic organisms and thus constitute potential public health hazard.

REFERENCES

Adebayo-Tayo, B. C., Odu, N. N., Anyamele, L. M., Igwiloh, N. J. P. N., & Okonko, I. O. (2012). Microbial quality of frozen fish sold in Uyo Metropolis. *Nature and Science*, 10(3): 71-77. https://www.sciencepub.net.

Baek, E., Lee, D., Jang, S., An. H., Kim, M., Kim, K., & Ha, N. (2009). Antibiotic resistance and assessment of food-borne pathogenic bacteria in frozen foods. *Archives of Pharmacal Research*, 32(12): 1749-1757. https://doi.org/10.1007/s12272-009-2213-y.

Bhaisare, D. B., Thyagarajan, D., Churchil, R. R., & Punniamurthy, N. (2014). Bacterial pathogens in chicken meat: review. *International Journal Life Science Research*, 2(3): 1-7. www.reseearchpublish.com.

Clinical and Laboratory Standards Institute (CLSI), (2018): Performance Standards for Antimicrobial Susceptibility Testing; *Twenty-Fourth Informational Supplement.* CLSI document M100-S24. Wayne, PA 34(1): 261.

Elhadi, N. (2014). Prevalence and antimicrobial resistance of Salmonella spp. in raw retail frozen imported freshwater fish to Eastern Province of Saudi Arabia. *Asian Pacific journal of tropical biomedicine*, 4(3): 234-238. https://doi.org/10.1016/S2221-1691(14)60237-9.

Ismail, K., & Belma, D. (2002). Microbiological investigations on some of the commercial frozen meat in Izmir. Turk. *Elec. J. of Biotech*, 35(1): 18-23.

Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H. (2005). Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *Journal of Veterinary Medical Science*, 67(1): 107-110.

Lalitha, M. K. (2004). Manual on antimicrobial susceptibility testing. *Performance standards for antimicrobial testing: Twelfth Informational Supplement*, 56(23): 454-459.

Noor Uddin, G. M., Larsen, M. H., Guardabassi, L., & Dalsgaard, A. (2013). Bacterial flora and antimicrobial resistance in raw frozen cultured seafood imported to Denmark. *Journal of food protection*, 76(3): 490-499.

Nyenje, M. E., & Ndip, N. (2013). The challenges of foodborne pathogens and antimicrobial chemotherapy: A global perspective. *African Journal of Microbiology Research*, 7(14): 1158-1172. https://doi.org/10.5897/AJMRx12.014

Okonko, I. O., Ogunjobi, A. A., Fajobi, E. A., Onoja, B. A., Babalola, E. T., & Adedeji, A. O. (2008). Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria. *African Journal of Biotechnology*, 7(16): 23-30. https://academicpress.com.

Oranusi, S. U., Obioha, T. U., & Adekeye, B. T. (2014). Investigation on the microbial profile of frozen foods: Fish and Meat. *International Journal of Advanced Research in Biological Sciences*, 1(2): 71-78.

Zakki, S.A., Qureshi, R., Hussain, A., Ghias, W., Sharif, M. and Ansari, F. (2017). Microbial quality evaluation and prevalence of bacteria and fungus in different varieties of chicken meat in Lahore. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, 5(1): 30-37.