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EVALUATION OF MICROBIAL PROFILES OF SELECTED READY AND NON-READY TO CONSUME FISH VENDED IN SOME OPEN MARKET IN AWKA, ANAMBRA STATE, NIGERIA

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ABSTRACT

Food Vendors play an important role in the cities and towns of many developing countries in meeting the food demands of the urban dwellers. It feeds millions of people daily with a wide variety of fish and fishery products that are relatively cheap and easily accessible. However concerns have been raised about the safety and quality of fish vended in open markets, Hence this study was carried out to determine the microbial profile and the hygienic and sanitary practices of fish vended in some open markets in Awka, A total of four fish samples in different forms comprising of ready and non - ready to eat (hot smoked, cold smoked, frozen and barbequed) were collected from some open markets in Awka such as Eke Awka, Second Market and Abakiliki Street. A number of microorganisms and fungi spp were isolated and identified using standard methods. However, Frozen and Barbequed fish were observed to have the highest number of microbes with their mean Total Bacteria Count (TBC) 6.35 $\times 10^5$ and "Too Numerous To Count" (TNTC) respectively and the lowest was the cold smoked fish with a mean TBC of 2.30×10^5 .The overall hygienic status of the vending environment and the sanitary condition and handling practices of the vendors were not to the standard. The results emphasized the microbial contamination of the fishes. This study recommends training of vendors on hygiene and sanitary standard in order to reduce microbial contamination of fishes.

***Keywords**: Microbial Quality, Open market, Fish, Disease, Processing*

INTRODUCTION

The demand for fish and seafood products is growing, making fish and seafood products an important commodity in the global food trade. Fish accounts for 60% of the world's protein supply, and 60% of developing nations obtain more than 30% of their animal protein from fish [1]. Fish is widely accepted throughout the nation due to its distinct flavor, taste, and good texture [2]. Fish contamination can be caused by personnel errors, raw material leaks, openings in buildings, and pet accidents. Some pathogens can even establish themselves in processing plants from niches where they can survive for an extended period of time. The safety, wholesomeness, and acceptability of fish and fish products for customers, as well as the management of microorganisms, are paramount in achieving the goals of public health authorities, food processors, and consumers regarding the quality of our fish[3]. The majority of fish that is sold in open markets in Awka, Anambra state is either ready to eat (smoked or barbecued) or not ready to eat (iced or frozen). This means that the fish is exposed to dust, contamination, and high temperatures, which promote spoilage and the growth of microorganisms that could lead to increased pathogen prevalence and transmission. Furthermore, bad personal hygiene practices, including not washing your hands after handling contaminated objects, promote the growth of harmful organisms in fish.

Since fish processing facilities are thought to be the primary source of pathogen introduction into fish and fish products, the majority of investigations concentrated on human pathogens there [4]. Specifically, L. monocytogenes has undergone a great deal of screening because of its environmental ubiquity, ability to grow at freezing temperatures, and serious health risks to humans. The origins of L. monocytogenes infection in seafood processing facilities have been examined by Jami et al. [5] since 1999.While, Fernandes *et al.* [6] discussed the contamination of fish and fish products with Salmonella in the fish production chain. They suggested that biofilm formation and inadequate sanitation contribute to the cross-contamination of human pathogens. In recent years, greater attention has been paid to the prevalence and persistence of spoilage microorganisms in the postharvest environment; contamination and cross-contamination at the consumer and restaurant levels; and temperature abuse during transportation and in the supply chain [7]. Postharvest contamination sources include fish contact surfaces (such as slicers, conveyor belts, and knives), non-fish contact surfaces (such as floors, drains, and walls), personnel (such as aprons, gloves, and boots), and others (such as air, ice, and water). A variety of postharvest processing and handling techniques collectively play a significant role in the safety of fish products.

Although they are typically thought of as safe, wholesome, and advantageous goods, fish have occasionally been linked to certain food safety problems [8], which pose a severe threat to society and would be harmful if left untreated. Fish is widely accepted as a good source of animal protein [9], but eating fish can also increase your risk of contracting diseases from infection or intoxication [10], some of which are specifically linked to pathogens that are resistant to antibiotics. This is extremely dangerous and will not help the country's aquaculture industry grow. Direct surface contact between microbial contamination on environmental surfaces and food products is possible, as is the transfer of microbial contamination by vectors like staff, pests, air movements, or cleaning procedures [11]. When fish is landed and is handled carelessly during stowing and cutting, bacteria may also enter the fish from the outside. Ice and salt are two common external sources of bacterial contamination; smashed ice is particularly known to harbor high bacterial loads[12]. The intestines, gills, and skin/slime of living and recently caught fish are home to microorganisms. Since the slime that covers fish's surface has been found to include a wide variety of bacterial taxa, the microbial ecology of fish depends on the microbial composition of the water in which they dwell [13]. Therefore, eating fish that has been taken from contaminated water raw or improperly cooked carries a number of risks, and fresh fish is especially prone to spoiling quickly due to its high microbial load[14]. Fresh fish preservation therefore becomes crucial. This can be accomplished by freezing, sun-drying and smoking, canning, etc., but even with these methods, it is still discovered that these fish contain a significant number of germs that the consumers are unaware of [14, 15].The goal of this study was to examine the microbiological quality of a sample of ready-to-eat and non-ready-to-eat fish as well as the degree of hygienic practices upheld by handlers when processing and storing smoked fish that is sold in open markets in Awka, Anambra state.

MATERIALS AND METHODS

Sample Collection

Four fish samples in different forms (**Plate1-4**) comprising of ready and non -ready to eat (hot smoked, cold smoked, frozen and barbequed) were collected from open markets in Awka such as Eke Awka, Second market, Ifite, Abakiliki street, Awka. Fish samples, frozen fish, hot smoked fish, cold smoked and barbecued fish were collected from different retailers at different locations at open market ,aseptically using sterile containers. They were then transported to Springboard Microbiological Laboratory, Awka, Anambra for the analysis.

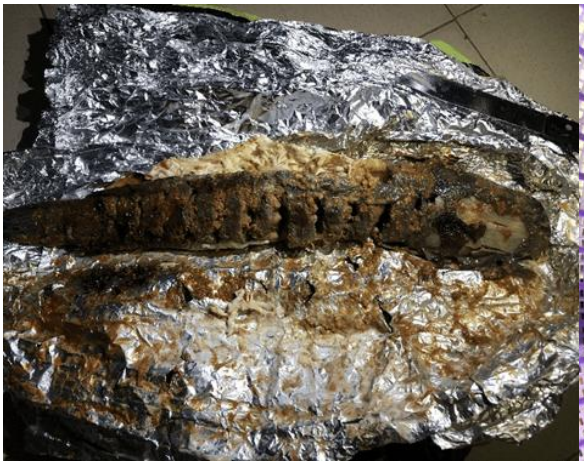

Plate1: Barbequed Fish

Plate 2: Hot Smoked Fish

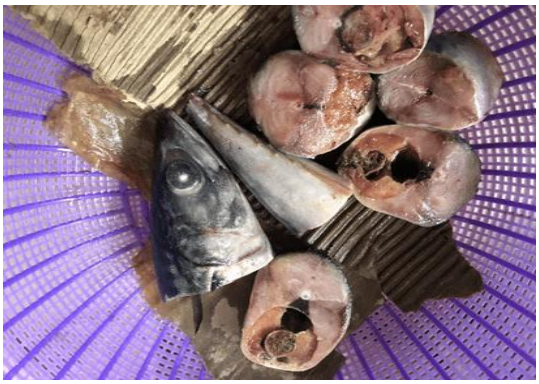

Plate 3: Frozen Fish

Plate 4: Cold Smoked Fish

Sample Analysis

One gram (1g) of the fish samples were weighed out aseptically and introduced into 10ml of sterile peptone water for bacteria, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10-2) of the sample were inoculated by plate method into sterile plates of Nutrient agar (NA),Eosin-Methylene Blue Agar (EMB), MacConkey Agar, Mannitol Salt Agar (MSA) and Salmonella-Shigella agar (SSA) plates for the culture of bacteria. The culture plates were incubated at 37°C aerobically for 24-48hours for bacteria; while Saboraud Dextrose Agar (SDA) was used for fungi. Developing colonies on Nutrient agar for bacteria and SDA for fungi were counted to obtain total viable. Discrete colonies for the bacteria were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods.

Total Bacterial Count (TBC) and Total Fungal Count (TFC) were calculated thus:

$$TBC/TFC = \frac{(N) \times 10}{VD}$$

Where TBC: Total Bacterial Count
TFC: Total Fungal Count
TCC: Total Coliform count
V: Volume plated
D: Dilution Factor

Characterization and Identification of Bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests [16]. The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition [17].

Gram Reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolorized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

Catalase Test
Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Motility Test (Hanging Drop Method)
A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interprete "drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile.

Oxidase Test
All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.Whitman No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observe the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). Positive was indicated by development of dark purple color (indophenols) within 10 seconds. Negative: Absence of color.

Urease Test Using Christensen's Urea Agar
The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive Protease from other Enterobacteriaceae.
Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire Christensen's Urea Agar slant surface. Adequate care was taken not to stab the butt as it will serve as a color control. The tubes were incubated loosened caps at 35 C. The slants were observed for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production would be indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive Proteeae (*Proteus spp., Morganella morganii, and some Providencia stuartii strains*) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella or Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative

Indole Test
A loopful of an 18-24 hour culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation was done at 35–37℃ first for 24 hours and further for up to 48 hours. Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

Methyl Red Test
Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

Voges-Prausker Test
Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was asceptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

Citrate Test
A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

Fungal Identification

Isolation and Characterization of the Fungi.

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the slide culture and lacto phenol cotton blue wet mount staining techniques for microscopic evaluation under X100 and X40 magnification of the microscope; with reference to the Manual of Fungal Atlases (Frey et al., 1979; Barnett and Hunter, 2000; Watanabe, 2002; Ellis et al., 2007).

RESULTS

The result presented in Table 1 shows that the fish sample BQ (Barbequed fish) has the highest bacteria count which was too numerous to count, followed by FF (frozen fish) with a mean TBC of 6.35×10^5, HS of 3.30×10^5 and CS of 2.50×10^4. Using nutrient agar medium, Table 1 also shows the total fungal count using SDA, Only one specie of fungi was identified which *is Candida sp*. The fish sample FF has the highest number of *Candida sp* with a mean TFC of 5.60×10^5 while BQ and HS (Hot smoked) have the same mean TFC and CS (Cold smoked) fish did not show any TFC. All the fish samples analysed have Candida sp while CS did not have any specie of fungi present.

Table 2 shows the result obtained from MSA(Mannitol Salt Agar),EMB (Eosine methyl blue agar) and Mac Conkey Agar to identify and count various organisms discovered in the fish sample .Using MSA, no bacteria was found in Hot smoked , cold smoked fish and Barbequed fish , while FF was shown to contain a bacteria which was identified as *Staphylococcus aureus*. The types of bacterial and fungal isolates in both fresh and smoked fish samples are similar to those previously observed in other studies by Akinjogunla *et al*.and Wogu and Maduakor.

Using EMB AGAR *3 E.coli* was identified in HS, *37 E.coli* and 3 *Proteus mirabilis* in FF and *17 E. coli* in BQ. Using this medium nothing was found in CS fish sample.Using MacConkey agar bacteria present in this medium was counted and identified; 2 *Enterococcus faecalis* and 1 *Salmonella typhimurium*, present in HS, in FF we identified *24 Enterobacter aerogenes* and *1 Enterococcus faecalis* and in BQ there was *13 Enterobacter aerogenes* and *35 Salmonella typhimurium*, while CS had no organism present.In Table 3, various tests using reagents were carried out to isolate and identify various bacteria in the fish samples .In Table 3, their forms, margin, elevation, surface, opacity and colours were also identified. Sugar fermentation tests was also conducted in order to identify sugars that can be fermented by bacteria which also aided us in identifying various bacteria. Table 4 shows the result using microscopy in which a cream coloured yeast like organism was observed and the shape viewed was spherical with a size of 2-6×3-7µm.The specie identified was *Candida sp.*

Table 1: Total Microbial Count and Diversity of Bacteria Isolated from the fish

SAMPLE	NUTRIENT AGAR			SDA			Identity of Fungi on SDA
	X	Y	Mean TBC	x	y	Mean TFC	
HS	32	34	$3.30x10^5$			$5.50 x10^4$	*Candida spp*
CS	2	3	$2.50 x10^4$	Nil	Nil	Nil	Nil
FF	62	65	$6.35 x10^5$			$5.60 x10^5$	*Candida spp*
BQ	TNTC	TNTC	TNTC	4	4	$5.50 x10^4$	*Candida spp*

Key:TNTC- Too Numerous To Count, ;HS- Hot-smoked (Ready to eat) ; CS- Cold-smoked (Ready to eat)
FF- Frozen Fish (Non-Ready to eat); BQ- Barbeque (Ready to eat) ;TBC- Total Bacteria Count
TFC- Total Fungal Count; SDA- Sabouraud Dextrose Agar; X – First Count of the bacteria/fungi
Y – Second Count of the bacteria/ fungi.

Table 2: Counts and Identities of Various Organisms found on the Fish Samples

SAMPLE	Mannitol Salt Agar		EOSINE METHYLENE AGAR		MacConkey Agar		Samonella Shigela Agar	
	N	Identity	N	Identity	N	Identity	N	Identity
HS	Nil	Nil	*3*	*Escherichia coli*	2	*Enterococcus faecalis*	1	*Salmonella enterica*
					1	*Salmonella*	1	*Shigella flexneri*

						typhimurium		
CS	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
FF	1	*Staphylococcus aureus*	*37*	*Escherichia coli*	24	*Enterobacter aerogenes*	3	*Salmonella enterica*
			3	*Proteus mirabilis*	1	*Enterococcus faecalis*	25	*Shigella flexneri*
BQ	Nil	Nil	*17*	*Escherichia coli*	13	*Enterobacter aerogenes*	2	*Salmonella enterica*
					35	*Salmonella typhimurium*	14	*Shigella flexneri*

Table 3: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Form	**Surface**	**Colour**	**Margin**	**Elevation**	**Opacity**	**Gram**	**Cat**	**Mot**	**Ind**	**MR**	**VP**	**Cit**	**Lac**	**Glu**	**Suc**	**Fru**	**Mal**	**Oxi**	**Ure**	**Identity**
Circular	Glistening	Cream	Entire	Raised	Transparent	- Rod	+	+	-	+	+	+	+	+	+	-	+	-	+	*Proteus mirabilis*
Circular	Smooth	Yellowish	Entire	Raised	Opaque	+ cocci	+	+	-	+	-	-	AG	AG	A	A	AG	-	+	*Staphylococcus aureus*
Circular	Smooth	Greyish/ Colourless	Entire	Convex	Translucent	- Rod	+	-	Var	+	-	-	-	+	-	+	var	-	-	*Shigella flexneri*
Circular	Smooth	Greyish/ white	Lobate	Low convex	Translucent	- Rod	+	+	-	+	-	+	-	+	-	+	+	-	-	*Salmonella enterica*
Circular	Smooth	Cream	Entire	Convex	Opaque	+coccus	-	-	-	-	+	-	+	+	+	-	+	-	-	*Enterococcus faecalis*
Circular	Smooth	Whitish	Entire	Convex	Translucent	- Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*Escherichia coli*

Gram: Gram reaction; **Cat**: Catalase test; **Mot**: Motility test; **Ind**: Indole test; **MR**: Methyl-red test; **VP**: Voges-Proskauer test
Cit: Citrate Utilization test;
Sugar Fermentation Tests:
Lac: Lactose Fermentation; **Glu**: Glucose Fermentation; **Suc**: Sucrose Fermentation; **Fru**: Fructose Fermentation
Mal: Maltose Fermentation '**Oxi**: Oxidase **Ure**: Urease

Table 4: Colonial and Microscopic Identifications of the Fungal Isolate

Colony morphology	**Microscopy**	**Identity**
Colonies (SDA) white to cream-coloured smooth, glabrous yeast-like.	Spherical to ellipsoidal budding blastoconidia, 2-6 x 3-7 µm in size. Ascospores may be produced on 5% malt extract or cornmeal agar after 5-30 days at 25OC.	***Candida sp.***

DISCUSSION

The current study examines the microbiological profiles of specific ready-to-eat and non-ready-to-eat fish that are sold in Awka, Anambra state, open markets. The study's findings showed that, with the exception of cold-smoked salmon, all of the fish samples contained the fungus Candida sp. It's possible that vendors handled the fish samples properly or that handlers followed excellent hygiene practices because there were no fungus in the fish samples. This is consistent with the observation made by Nwiyi and Onyeabor [18] that a higher rate of spoiling is caused by harsh handling. Similar to Hassan's findings [19], the fungal contamination of these fish samples may have resulted from poor fish handling, storage, marketing, and transportation practices. Seven bacterial species were also identified in this study: Shigella flexneri, Proteus mirabilis, Salmonella sp., E. Coli, Enterococcus faecalis, and Staphylococcus aureus. One of the main infections linked to fish deterioration after harvest is Escherichia coli. This result is consistent with earlier research by Gram and Huss [20], who found that the microbial count on various media indicates contamination and that this organism is one of the main sources of microbial deterioration of fresh fish after capture. On the other hand, Emikpe et al. [1] found in a related investigation that different food spoilages could be caused by enteric bacteria originating from humans and animals contaminating fish.

Since Escherichia coli may be found in practically every environment, including human skin, water, and air during processing, the reason it was present in every sample could be explained by its ubiquity. This conclusion supported the

findings of Majeed and MacRae [20], who found that Salmonella and E. coli were the most common Gram-negative rod bacilli linked to fish rotting. In addition, frozen fish had a higher E. Coli value than the other three fish samples. This is most likely because the moisture content of frozen fish is higher than that of the other samples. Enterobacteriaceae and other thermosensitive bacteria are utilized as markers of poor hygiene and post-cooking food contamination [22]. The presence of E. Coli in samples (HS, FF, and BQ) further indicated the possibility of animal or human fecal matter contamination as a result of inadequate adherence to hygiene protocols, which has been identified as one of the primary causes of contamination in this investigation. The hawkers' lack of hygiene is indicated by the presence of coliform, which may also be the result of fecal contamination[23].

Because these bacteria carry virulent genes, the presence of E. Coli, Enterococcus spp., Salmonella spp., and Staphylococcus aureus is concerning for public health. Staphylococcus aureus suggests that the fish may have been contaminated at the source due to incorrect handling procedures used by processors. 20% to 50% of healthy persons may have S. aureus, which is a common habitant of the skin and mucous membranes [24]. Food contamination may result from careless handling and poor hygiene, which may ultimately have an impact on customers' health. Human interaction during handling and processing is responsible for the typical flora of human skin and mucous membranes, including Staphylococcus aureus [25]. In addition to producing a range of extracellular enzymes and toxins that have been linked to food poisoning, Staphylococcus aureus can quickly become resistant to several antimicrobial drugs and present therapeutic challenges[26]. It has long been known that Staphylococcus aureus is one of the main pathogens that infect humans and causes sickness.

Food poisoning may result from consuming fish or fish products that contain this. Staphylococcus aureus may be introduced as a result of the measures used to maintain and protect the quality of these fish samples during their transportation to different stores or retailers and until they reach the ultimate customer. Because Staphylococcus enterotoxin is heat stable and resistant to gastrointestinal enzymes, Okareh and Erhahon [27] also confirmed that Staphylococcus sp. are pathogenic strains that may cause food poisoning. According to Morales et al. [28], workers who are carriers of Staphylococcus aureus or who come into touch with poorly cleansed equipment are typically responsible for food contamination. One of the infections found in the frozen fish was S. aureus, which is normally present in human flora but not in fish. Its presence in the samples may have resulted from environmental and staff contamination [29]. The fish samples' presence of Salmonella sp. suggests that the processing water was tainted by human waste. Typically, Salmonella sp. is not a pathogen that affects fish; instead, it is an infection that results from consuming feed and water tainted with Salmonella. When raw or undercooked fish is consumed, this pathogen can cause bacteremia, cramping in the abdomen, diarrhea, and enteric fever in humans, especially in the elderly or very young[30]. The third prevalent bacteria found in this investigation was Shigella flexneri; it is gram negative and ferments glucose rather than lactose.They can be found in water, and tainted water can spread them.It results in nausea, headaches, fever, and diarrhoea.

Enterobacter aeorogenes is a direct indicator of environmental and faecal pollution caused by animals, humans, or careless handling techniques. Fish sample FF had the opportunistic pathogen Proteus mirabilis, whose presence may have resulted from faecal contamination. It mainly affects people with weakened immune systems; even though there were not many Enterococcus faecalis present, this still suggests that the processing water was tainted with feces. The analysis's findings corroborate those of [31], who examined smoked salmon that had been prepared in Benin microbiologically. The fish samples had total fungal counts ranging from 5.50×104 to 5.6×105 CFU/g. This indicates that all the fish under evaluation were confirmed as being of good quality by microbiologists, and therefore, the counts were within the allowable range of the international norm. The approved threshold of <100 MPN/g for the total coliform count in frozen fish is set by ICMSE (1986). According to the data from this investigation, the total counts of bacteria from the fish samples ranged from 2.50×104 to 6.35×104 CFU/g. It is widely acknowledged that fish that have a microbial load greater than 106 CFU/ml are probably at a stage where they are unsafe for human consumption and unsatisfactory from a microbiological standpoint [32].

Since each of these species has a unique manner of altering the health conditions of consumers of such contaminated fish, the overall bacterial count on fish rarely indicates the quality of the fish but rather the danger of spoiling induced [33]. Furthermore, samples of frozen, barbecued, and hot-smoked fish had noticeably increased bacterial and fungal loads. Thatcher and Clark [34] have previously reported on the types and quantities of hygienic measures taken during the handling and smoking procedures. As previously noted by Dike-Ndudim et al.[35], the cause may also be a sign of partial dehydration from smoking and unclean displays at market stalls. The primary source of this contamination is that the vendors never sought out nor received any official training in food preparation. Fish handlers should possess the knowledge and abilities needed to handle food in a hygienic manner, according to FAO [36]. Mechanisms for keeping food handlers informed about all the steps required to preserve food safety and appropriateness should be implemented. According to FAO [37], before receiving a license, all food vendors and assistants should complete a basic food hygiene training course.

CONCLUSION AND RECOMMENDATIONS

The results of this investigation showed that the non-ready-to-eat (frozen) fish and ready-to-eat (cold, hot, and barbecued) fish sold in outdoor markets in Awka, Anambra state, are not always of adequate microbiological status and may be sources of foodborne illnesses. The substandard microbiological quality of these items is caused in part by inadequate handling procedures during processing and sales, inadequate hygiene standards, and low-quality raw materials. To produce a safe and healthy product for consumption, processors and sellers should get training on proper cleanliness and handling techniques.Food poisoning and spoiling of fish samples are caused by the presence of these

microbes. Microbes were isolated from the fish samples, indicating that inadequate fish preservation may result in labor losses for sellers and health hazards for customers if the fish is not handled and processed appropriately before consumption.as a crucial element. The pathogenic bacteria identified from the fish highlighted the possibility of fish serving as a vehicle for human infection, even though the microbial load reported from the fish is low and acceptable. Fish is a daily staple of every Nigerian home's diet. There is currently no literature or data on illness outbreaks linked to the consumption of ready-to-eat or unready-to-eat fish. The consumed fish are either in the form of dried, smoked, boiled or fried, or barbecued.In addition to being nutrient-balanced, fish should also be microbiologically safe. Good hygiene standards, such as using Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing standards (GMP) throughout the fish production and processing chain, can help avoid foodborne disease. According to WHO and FAO guidelines, the government should impose strict rules on food safety, with a maximum allowable microbial load of 106 CFU/ml for fish. Additionally, sellers must receive training on hygienic procedures and food safety.

These recommendations are based on the study's findings and include the following:

• To minimize potential contamination, it is important to stress the importance of microbiological examination of fish.

• Fish must be stored at specific temperatures to decrease the growth of any pathogens that may be present in the ready-to-eat or unready-to-eat fish, in order to prevent the formation of toxins in the fish.

•When handling and processing fish, caution should be used.

• Reduce microbiological contamination by increasing knowledge of the potential health risks.

• It is advised that fish mongers get education regarding the significance of upholding high standards of hygiene and the use of treated water while processing fish.

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