Original Research Article

Microbiological Assessment of Smoked Fishes From Various Processing Points in Ado Ekiti Metropolis

Abstract

The aim of the research is to isolate and characterize the bacteria present in smoked fish sold in various processing site in ado Ekiti Metropolis.Fifty (50) samples of five (5) species of fish (Mackerel:*Somber scomber,* Herrings: *Clupea harengus,* Horse mackerel: *Trachurus trachurus,* Blue whiting: *Micromesistius poutassou,* Catfish: *Clarias gariepinus*) were obtained from fifteen (15) different processing site in different location in Ado-Ekiti. The study results showed the total heterotrophic bacteria count which ranges from 0.30 $x10^4$ – 3.68 x 10^4 the highest colony count was examined from the panla (Blue whiting {*Micromesistius poutassou*}.The bacteria organisms isolated and identified in the smoked fish were *Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus sp, Bacillus subtilis, Escherichia coli, Proteus mirabilis and Staphylococcus aureus.*The study showed that though smoking helps in inhibiting activities of bacteria, however, when not properly carried out, bacterial growth and activities still lead to the deterioration of the fish. Due to public health implication, the state of smoked fish should be paid proper attention by the processors and consumers for their safety through proper processing, storage and handling procedures.

Keywords : Fish processing, Bacteria,

Introduction

Fish plays an important role in fighting hunger and malnutrition. Fish is not only a source of proteins and healthy fats, but also a unique source of essential nutrients, including long-chain omega-3 fatty acids, iodine, vitamin D, and calcium. The multiple benefits of fatty fish high in omega-3s and small fish eaten whole containing nutrients in the skin and bones clearly illustrate seafood's irreplaceable nutritional value. An increased focus on fish and nutrition aids both developing countries and the developed world. In many developing countries, fish is the main or only source of animal protein, and is essential for providing micro nutrients to vulnerable populations. Fish can sometimes serve as a solution to existing health problems. For instance, goiter is found in areas where iodized salt is unavailable, but

the consumption of fish and the natural iodine it contains could help reduce these cases. Dietary patterns are also shifting in developed and middle-income countries, and an increasing emphasis on coronary and overall health has led to an increased demand for fish (Robert and Stadler, 2000).

Fish smoking is one of the traditional processing methods aimed at preventing or reducing post-harvest losses. Fish is a highly perishable product due to its high susceptibility to autolysis, oxidation and hydrolysis of fats and microbial spoilage. The post-harvest methods of preserving fish include refrigeration (4ºC) and freezing which is effective when such fish is conditioned to a temperature of -10ºC. Other methods frequently employed include sun drying and smoke drying all associated with increased germicidal action with increasing temperature. Fish is one of the most important animal proteins available in the tropics, and it represents about 14% of all animal proteins in a global basis. In Nigeria, the demand for fish consumption is increasing due primarily to health benefits of eating fish and secondarily to increase in human population and the rinderpest disaster and drought bane which reduce the availability and affordability of red meat (cattle, sheep and goat) (Al-Harbi and Uddin, 2005).

Fish is an extremely perishable food immediately after catch and therefore requires immediate and proper handling and good preservation to retain its quality. The food eaten has a direct influence on health, it is therefore an important task that food inspectors, food manufacturers and food handlers keep food safe from pathogenic microorganisms, especially when such foods are to be consumed without further processing, i.e. fast foods or "ready to eat" foods. Several types of microorganisms have been known to affect the quality of food, thereby constituting health hazards when food contaminated with these organisms are consumed. A number of food items sold locally have been shown to be highly contaminated with *Bacillus species, Staphylococcus* species, and other bacteria species (Oronsaye *et al.*, 2010).

Microbial contamination is caused by microorganisms known as bacteria. Bacteria are found almost everywhere in the environment including soil, water, plants, animals and humans (Baird-Parker, 2000). The main carriers of bacteria are the foods that we consume which are rarely sterile (Adams and Moss, 2000). Food carries microbial associations whose composition depends on the organisms' access to food, their ability to grow and the interaction in the foods as time progresses. Moreover, the exact origin of bacterial contamination in food depends on the natural microflora of the raw material and those

organisms introduced in the course of harvesting, slaughter, processing, storage conditions and the distribution of food (Adams and Moss, 2000). Microbial contamination cause infections that are responsible for various food borne illnesses and diseases. In uncontrolled conditions, these infections could cause major foodborne disease outbreaks in a community or population. Espejo-Hermes (1998) has identified that infections caused by these microbes are more pronounced in developing countries, where there are improper practices in handling, storage, processing and distribution of food and food products.

Aims and Objective if the study

I. To carry out a qualitative investigation on the occurrence of microorganism in fish sold at various processing points in Ado-Ekiti metropolis.

II. To identify pathogenic present in fish samples obtained from processing point using various biochemical tests.

III. To compare the incidence of these pathogens at different processing point of the fish samples and to determine whether the various points have any significant effect on the prevalence of these pathogens in the fish.

MATERIALS AND METHODS

Materials

Petri-dishes, paper tape, distilled water, cotton wool, methylated spirit, microscope, hand glove, respiratory mask and distilled water.

Collection of Fish Sample:

Fifty (50) samples of five (5) species of fish (Mackerel:*Somber scomber,* Herrings: *Clupea harengus,* Horse mackerel: *Trachurus trachurus,* Blue whiting: *Micromesistius poutassou,* Catfish: *Clarias* gariepinus) were obtained from fifteen (15) retailer shops selling at road side in different location in Ado-Ekiti. The samples were collected in a sterile foil paper and put inside ice cooler and transported to Microbiological laboratory in Federal Polytechnic Ado-Ekiti.

Preparation of media

The media used was nutrient agar (NA), which were prepared according to manufacturer instructions. The media was dissolved in adequate amount of distilled water. The media was homogenized and autoclaved at 121^0C for 15minutes.

Isolation of microorganisms

10g of the fish sample were weight and macerated using mortar and pestle, 1g of the macerated sample was taken and dissolved inside 10ml of distilled water.

A series of test tubes were prepared for the isolation of bacteria and fungi. 9mL of sterile distilled water was put into each of the test tubes. To the first test tube, 1mL of the sample was added to give a dilution of 10^{-1}. The contents were shaken properly and 1mL of the solution was taken and added to the next test tube containing 9mL of sterile distilled water to make a concentration of 10^{-2}. The serial dilution was made up to 10^{-6} dilution for the fish samples. 0.1ml of the 10^{-3} and 10^{-4} dilution was cultured on the agar plates using the pour plate technique (Fawole and Oso, 2001) labeled and incubated at 37^0C for 24hours. Counting of the bacterial colony was done by using colony counter, distinct colonies were sub cultured picked by streaking on fresh nutrient agar plates. The pure culture was preserved on agar slants for further studies.

Biochemical tests

Gram staining technique

Smear of each bacterial isolate was prepared on a clean slide. In preparing the smear, a drop of sterile distilled water was placed in the middle of the slide. A sterilized inoculating needle was used to pick from the bacterial colony and rubbed on the slide containing a drop of sterile distilled water. The bacterial cells were spread into a thin smear, air dried and heat fixed.

Catalase test

A thick emulsion of each test organism was prepared on a clean slide. Several drops of 3% hydrogen peroxide were added on each of the slides. A positive result was indicated by effervescence which was caused by the liberation of oxygen gas as a result of catalase production by the bacterium. There were no gas bubbles in the bacteria that do not produce catalase

Coagulase test

A loopful of test isolates were picked from a young culture, emulsified with serum placed on a clean grease free slide and rocked for one minute. The presence of agglutination indicates a positive reaction

Motility Test

The hanging-drop method was used to determine the motility of the bacterial isolates. A little Vaseline was placed around the edge of the hallow of a clean cavity slide. A loopful of each isolate was transferred to the center of a clean coverslip laid on the bench. The cavity slide was carefully inverted over the coverslip and the slide was pressed down gently in order to seal the coverslip with the slide. The unit was then inverted in such a way that the loopful of the bacterial colony was in hanging position. The preparation was examined immediately under the X40 objective lens. The microscopy was done quickly in order to avoid excessive illumination, which could quickly cause the organism under study to lose motility.

Motile cells came in view and were seen moving rapidly in the field while non-motile were not moving.

Antibiotic sensitivity test

A broth culture of each organism was prepared and a sterile cotton swab was inserted into the broth culture to pick the isolates, then the inoculums on the swab stick were transferred into a fresh plate of nutrient agar. Each of the organisms was classified as either Gram positive or Gram negative and the sensitivity disc of each was placed on each plate containing the isolated organism. The plates were incubated invertedly at 37^0C for 18-24hours.

RESULTS AND DISCUSSION

Results

Table 1: Total heterotrophic count of bacteria and fungi present in smoke fish purchase from different processing site in Ado-Ekiti metropolis

Sample code	Total Heterotrophic bacterial count (10^4cfu/g)
IIP	3.08×10^4
FMP	3.24×10^4
US	0.88×10^4
UA	0.52×10^4
AP	1.44×10^4

IMA	0.56×10^4
AJS	3.04×10^4
OIS	1.32×10^4
ERS	1.04×10^4
OBSP	2.04×10^4
ASAS	1.08×10^4
OJS	0.30×10^4
OAP	0.84×10^4
OWSEP	3.68×10^4
NRK	3.14×10^{-4}
OAC	3.40×10^{-4}

Key: IIP:- Isato Isale **Panla**, FMP:- Falegan Market Opposite Ikeja Avenue **Panla**, US:- Ureje **Sawa,** UA:- Ureje **Alaran,** AP:- Adehun **Panla**, IMA:- Irona Mark*et al***aran**, AJS:- Ajebandele Junction **Sawa,** OIS: Oke-Ila **Sawa,** ERS:- Egbewa Road 6 **Sawa,** OSP:- Okebola Street Life Bible Church **Panla,** ASAS: No 19 Adeike Street Ajilosun **Sawa,** OJS: Omisanjana Opposite Fountain Junction **Sawa,** Ori-apata **Panla,** Owolabi street Ekute **Panla,** Nova road **Kote** and Odo-Ado **Catfish**

This study shows that pathogenic bacteria were present in smoked fish sold in, Isato Isale, Falegan market, Ureje, Adehun, Irona, Ajebandele junction, Omisanjana, Egbewa, Okebola, Oke-ila and Adeike stre*et al*l in Ado-Ekiti. Table 1 shows the total heterotrophic bacteria count which ranges from $0.30 \times 10^4 - 3.68 \times 10^4$ the highest colony count was examined from the panla (Blue whiting {*Micromesistius poutassou*}) sample purchased from Owolabi street Ekute located in Ado-Ekiti, while Horse Mackerel: *Trachurus trachurus* purchase from Omisanjana opposite fountain junction has the lowest concentration of contamination. Also the fungi result was seen in table 1 which fish sample purchase from Isato isale has the highest heterotrophiv fungi count of 6.20×10^4 and the lowest was examine in the fish sample purchase from Odo-ado with 1.04×10^4 .

Table 2: Colonial morphology, cellular morphology and biochemical characteristics of the bacterial isolates from smoke fish sold in different processing point in Ado-Ekiti metropolis

Sample	Colony	Colony surface	Gram's Rtn	Colony shape	Catalase	Coagulase	Motility	Indole	Citrate	Oxidase	Spore	Methyred	V.P	Detected Organisms
IIP	G	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	***Pseudomonas aeruginosa***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
FMP	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
	C	Rough	**-ve**	Rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	***Escherichia coli***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
US	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
	G	Smo	**-**	Rod	+v	-	+v	-	+v	+v	-	-	-	***Pseudomo***

		oth	**ve**	s	e	ve	e	ve	e	e	ve	ve	ve	***nas aeruginosa***
	C	Rough	**-ve**	Rods	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	***Klebsiella pneumoniae***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
UA	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
	C	Rough	**-ve**	Rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	***Escherichia coli***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
AP	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
	Y	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	***Proteus mirabilis***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
IMA	G	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	***Pseudomonas aeruginosa***
	C	Rough	**-ve**	Rods	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	***Klebsiella pneumoni***

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AJS	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
OIS	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
ERS	G	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	***Pseudomonas aeruginosa***
	C	Rough	**-ve**	Rods	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	***Klebsiella pneumonia***
OBSP	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
ASAS	C	Rough	**-ve**	Rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	***Escherichia coli***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***

OAP	Y	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	***Proteus mirabilis***
	Y	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	***Proteus mirabilis***
	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
OWSEP	G	Smooth	**-ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	***Pseudomonas aeruginosa***
	C	Rough	**-ve**	Rods	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	***Klebsiella pneumoniae***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
NRK	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***
	C	Rough	**-ve**	Rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	***Escherichia coli***
OAS	Y	Rough	**+**	Cocci in cluster	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Staphylococcus aureus***
	C	Rough	**+ve**	Cocci in chain	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	***Streptococcus sp***
	C	Rough	**+ve**	Rods	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	***Bacillus subtilis***

Key: IIP:- Isato Isale **Panla**, FMP:- Falegan Market Opposite Ikeja Avenue **Panla**, US:- Ureje **Sawa,** UA:- Ureje **Alaran,** AP:- Adehun **Panla**, IMA:- Irona Mark*et al***aran**, AJS:-

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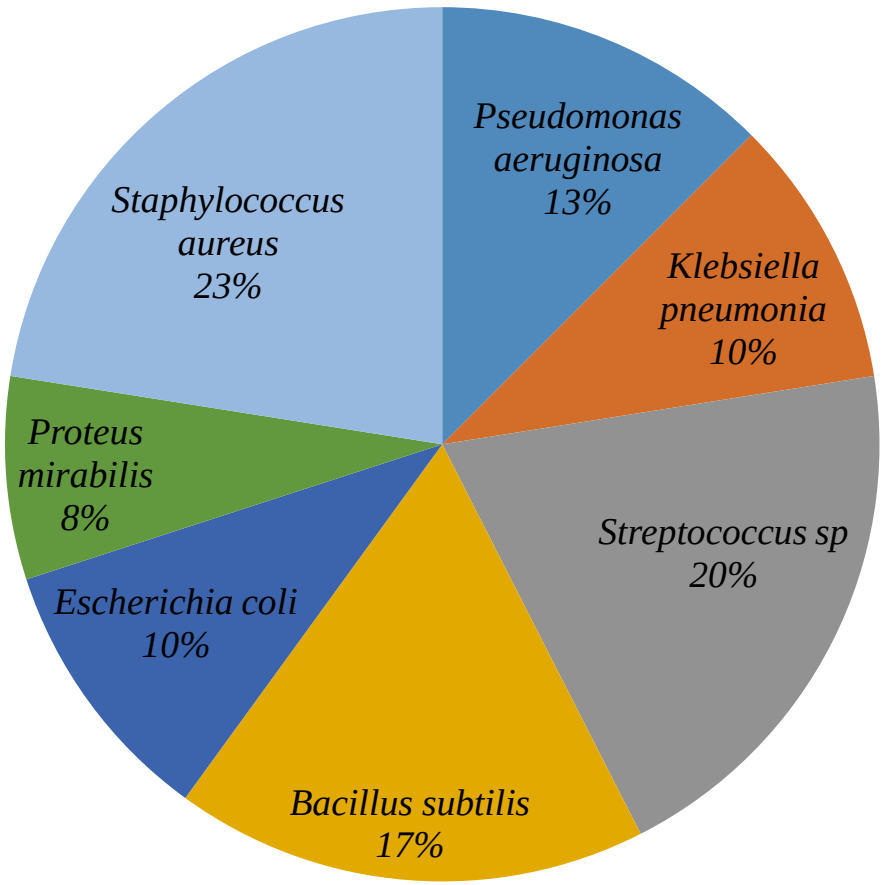
Positive = +ve , Negative = -ve, Y = Yellow, C = Cream, G= Green and S = Smooth

Six (7) different bacteria genera were identified in smoked fish sold in Ado-Ekiti, Ekiti State these were *Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus sp, Bacillus subtilis, Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus*

Seven (7) different bacteria genera were identified in the smoked fish sold in Ado-Ekiti, Ekiti State, these were *Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus spp, Bacillus subtilis, Escherichia coli, Proteus mirabilis and Staphylococcus aureus* as shows in table 2 with different biochemical test. Moreover, most of the organisms isolated in this study might have been introduced into these foods from water used for washing, utensil and wrapping materials, the exposures of the products to high temperature storage and unhygienic condition of handling, reheating of kept food and the open market which are heavily polluted by various microorganisms. The high microbial load may be due to the environmental condition and also due to the fact that the place is highly congested with traffic, which may create dust from which the fish may be contaminated.

Figures 1: Frequency occurrence of bacteria isolated from smoke fish sold in Ado-ekiti Metropolis



Among these isolate *Staphylococus aureus* is the more abundant (23%) followed by *Streptococcus* (20%), *Bacillus subtilis* (17%), *Pseudomonas aeruginosa* (12%), *Klebsiella pneumonia* and *Escherichia coli* both has (10%) respectively while *Proteus mirabilis* is the least isolate that has less occurrence

Table 3 shows the frequency occurrence of bacteria isolates which *Staphylococcus aureus* has the highest cumulative frequency, this result agrees with that of Brown; Okonta and Ekelemu (2005). *Staphylococcus spp*. is said to be one of the predominant bacteria that the smoked fish samples were contaminated with, this agrees with the work of Okonta and Ekelemu (2005) and Okonko *et al*., (2008) who reported *Staphylococcus* as one of the predominant bacteria contaminating smoked fish and causing spoilage. Its evidence would be as a result of poor sanitary condition and lack of adequate packaging of the products as they are always exposed at the market. These organisms may have contaminated the smoked fish through human handlers, air and soil. The bacteria group of *Staphylococcus spp*. according to Herman *et al*. (2011) reported that it was one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting in a diseased condition. This bacteria class may also cause superficial and systemic infections such as boils, impetigo and folliculitis while more serious and more common infections could be pneumonia, bacteremia and other infections of the bones and wounds as reported by Adelaja *et al*., (2013). In addition the findings of Moshood *et. al*., 2012 corroborates the findings in this study, since common bacteria such as *Staphylococcus aureu*s, and *Bacillus subtilis* were also isolated. The presence of these organisms in the smoked fish samples of *Clarias gariepinus* might be due to increase in moisture content of the product during storage, and also increase in temperature that favours the growth of these organisms.

Bacillus sp was also found to be present in the smoked fish samples which agrees with a similar study carried out by Moshood and Tengku Haziyamin (2012), *Bacillus aureus, Staphylococcus aureus, Proteus mirabilis, Klebsiella sp., Salmonella typhii* and *Streptococcus sp*. were all found to be associated with smoked fish. It is suspected that these organisms may have contaminated the smoked fish through human handlers, air and soil. The presence of these organisms in the started from the processor of the smoked fish might be due to increase in moisture content of the product during storage and also increase in temperature that favours the growth of

these organisms. All the pathogens are of food and public health implication and hence hazardous and injurious to human health, if consumed. Microbial load on ready-to-eat foods is important, however, factors such as, processing, storage and display may influence the microbiological load of ready-to-eat foods at the point of sale. Although smoke-drying reduces water activity and destroys bacteria through the agency of heat, post-processing contamination can and do occur especially during handling and transportation of processed foods to the point of sale.

The pathogenic state of species of *Streptococcus sp* is alarming this has become important disease agent in the aqua culture industries, Efuntoye *et. al*., (2012), reported that *Streptococcus sp* is the dominant etiological agent characterized by clinical symptoms, such as chronic wasting syndrome, heamorrhagic septicaemia, exophtaimia and meningitis with abnormal swimming. *Streptococcal* diseases have been reported worldwide in wild and farmed populations of diverse fresh water and marine fish.

The public health importance of bacterial and fungi of smoked fish species have not been adequately defined due mainly to mode of food preparation in the tropics, which involved cooking for considerable length of time. The heat would have eliminated most, if not all the bacterial flora. It is noteworthy that sanitary condition under which fishes are handled, processed and stored be improved upon to reflect standard or good practices.

Conclusion

The study showed that though smoking helps in inhibiting activities of bacteria, however, when not properly carried out, bacterial growth and activities still lead to the deterioration of the fish. Due to public health implication, the state of smoked fish should be paid proper attention by the processors and consumers for their safety through proper processing, storage and handling procedures. It is noteworthy that sanitary condition under which fishes are handled, processed and stored be improved upon to reflect standard or good practices.

In conclusion, there is need to introduce routine sanitary measures to control of contamination of food products with these microorganisms. High level of *Bacillus*

Sp, Coliforms and other pathogenic bacteria in food should not be dismissed as mere contamination, since they are capable of causing serious infections and food poisoning.

Recommendations

This study recommends that the fish handlers should be educated on proper hand washing during fish processing. Public education of the fish processors on the need for proper environmental sanitation. Good hygienic practice aimed at minimizing the microbial load of fish must be ensured. Greater attention should therefore be paid to the microbiological standard of the activities of roadside mark*et al*l the time. There must be good hygienic condition for food presented or market on the road side (smoked fish) such as using clean glass boxes, clean food wrappers to prevent excessive environmental contamination for smoked fish. The food handlers must observe basic sanitary rule on the smoked fish (food) as their direct consumption may pose a lot of health hazard to the life of man.

Standard organization should set standard/surveillance on the smoked fish. If these measures are put in place, they will go along way to significantly reduce toxin and contaminants foods, thereby reducing the health hazards to man.

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