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ISOLATION OF BACTERIA ASSOCIATED WITH SMOKED AND FRIED FISH SOLD IN OZORO

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Abstract:

Smoked and fried fish are largely consumed as a source of nutrient by man. It has been established that fish food can act as vehicle for transmission of some bacteriological pathogens especially in immune compromised individuals. Samples of fish were bought from different locations in Ozoro. The samples were labeled sample A and B (smoked fish) and sample C and D (fried fish). A total of five (5) bacterial species were isolated from the test samples: Vibrio chariae, Clostridium species, Proteus mirabilis, Lactobacillus species, and Corynebacterium species. The total heterotrophic bacteria count ranges from $4.8*10^3$cfu/ml to $40.0*10^4$cfu/ml. The mean plate count shows that smoked fish has the highest mean count $8.5*10^3$cfu/ml while fried fish has the least mean count $7.1*10^3$cfu/ml. Vibrio chariae have the highest percentage (%) occurrence (37.5%), while Lactobacillus species have the least percentage (%) occurrence (6.25%). The presence of the isolates in smoked and fried fish when consumed could induce gastrointestinal and metabolic disturbance.

Key words: *Isolation, Bacteria, Associated, Smoked, Fried, Fish*

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INTRODUCTION:

Fisheries play a significant role in rural African economy; earning livelihood for as much as 10% of the African rural population. The fisheries sector can play a significant role in creating new employment opportunities for Africa's rapidly expanding population. For every new job created in the primary fish production sector, another 3 to 5 jobs are consequently created in the post-harvest sector (Bernacsek, 2002).

Fish is one of the most valuable sources of food worldwide. People obtain about 20% of their animal protein from fin fish and shell fish (FAO, 2008) reported that about 35% of all fish is eaten fresh, chilled, or frozen. Fish and marine products are used as medicine, ground into vitamins or processed into cosmetics and perfumes, lubricants, varnishes soap and margarine Omega-3fatty acids are very important for normal growth particularly for the blood vessels and nerves and keeping our skin and other tissues youthful. Research studies have revealed that in population that consume large quantities of fish, with a high utilization of omega 3s, there is reduced risk of heart diseases (Ackman, 1992).

The methods of traditional fish processing in Ghana are smoking, salting, drying, fermentation, and frying (Nketsia-Tabiri and Sefa-Dedeh, 2000; Neequaye-Tetteh et al., 2002). Among these, smoking is practiced the most; it is estimated that more than 60% of the country's fish landings are preserved by smoking (Adu-Gyamfi, 2006). Historically, smoked fish has also been the most patronized of all traditionally processed fish in Ghana (Orraca-Tetteh and Nyanteng, 1978; Adu-Gyamfi, 2006). This high level of smoked fish processing and consumption is also true for other West African countries (UNDP, 2002).

Fish has associated bacteria pathogens that could be present in the skin, gills or gut. These organisms have been categorized into two groups: indigenous and non indigenous pathogenic bacteria (Nickelson and Finne 1992; Huss *et al.*, 1995).

The indigenous pathogenic bacteria are commonly found in the aquatic environment; they are present on the live fish and their presence in the final product is probable. They include *Listeria monocytogenes, Clostridium botulinum, Aeromonas hydrophila* and *Vibrio sp* (Huss *et al.*, 1995; Nickelson and Finne 1992). Non-indigenous pathogenic bacteria are normally associated with human or warm-blood animals and their faeces, and are not naturally present in fish. They are therefore contaminants. *Salmonella sp, Escherichia coli and Staphylococcus aureus* are among such pathogens (Huss *et al.* 1995; Nickelson and Finne 1992).

In Nigeria, fish alone contributes on the average 20-25% per caput animal intake and could be as high as 80% in coastal and riverine communities. (FAO, 1977) estimated the projected population and fish demand and supply from 1997 to 2025, with domestic fish population by the year 2007 as 0.77 million tones.

A decline in fish availability will have a detrimental effect on the nutritional status of the citizenry in places where fish contributes significantly to the protein intake of the people (Eyo, 2001). Fish losses arising from bacteria and autolytic spoilage are enormous. (Ibru, 1986) analyzed and enumerated some of the problems as inadequate modern inputs for fishing, poor and most often non-existing access roads and other means of communication between key production areas and marking as well as administrative centres, others are lack of processing, storage, distribution and marketing. A global study published in nature concludes that 90% of all large fishes have disappeared from world oceans in the past half century, the devastating result of industrial fishing (Wilson, 2001).

MATERIALS AND METHOD:

Study Area:

This research was conducted in Ozoro, Isoko North L.G. A of Delta state in Nigeria. Ozoro is situated along the high way between Ughelli and Kwale town. It serves as collection point of Cassava and palm oil. This location is chosen because it is a fast-growing community with high social activities being boosted by the presence of a tertiary institution (Delta State Polytechnic). It is densely populated and their major occupation is farming and trading

Sample collection:

Sixteen samples (Four samples each) from different market location in Ozoro, Delta State were used for the analysis. The samples were labelled A, B, C and D. Sample A (smoked fish from Ala square market), sample B (smoked fish from Akporie market), sample C (fried fish from Ala square market) and sample D (fried fish from Akporie market). The samples were placed in a sterile nylon at the place of collection. These were transported to the laboratory where analysis was carried out immediately.

METHODS:

Sterilization of Glass wares:

The glass wares that were used for this project were washed with detergent rinsed thoroughly and sterilized using autoclave at 121ºC for 15minutes.

Analysis:

Isolation of test organisms:

Each of the samples was serially diluted in six-fold according to Cheesbrough (2002). 0.1ml of the diluted samples was transfer in to sterile petri dishes. Prepared nutrient agar at very low temperature (temperature that can enhance the survival of the organism in the samples) was poured on the sterile petri disc containing the sample. The plates were incubated at 37ºc for 24 hours and subculture was carried out on the growth after 24 hours of incubation. Media prepared was according to the manufacturer instruction and then used for isolation of bacteria.

Pure isolates were identified according to their morphological characteristics and reactions to biochemical test.

Characterization and identification of isolates

The bacterial isolates were characterized and identified on the basis of their morphological characteristic and reaction to biochemical test, Catalase, Citrate, Indole and Triple sugar iron agar test were performed according to the scheme of Cheesbrough, (2002).

Morphological Characteristics

Gram staining:

Smear of each bacterial isolate was made on a grease free clean glass slide with a drop of normal saline, air dried and heat fixed by quickly passing the slides over flame. The smear was flooded with crystal violet for one minute (1 min) then wash, add lugol's iodine solution for 1minute and then washed with water which it was decolourized with 95% alcohol for 15secs and rinsed off with water again. The slide was then flooded with safranine red for one minute to counter stain and washed off with water, dried and examined under the microscope using oil immersion and x100 objective.

Biochemical Test

The biochemical analyses carried out were in accordance with procedures reported by Cheesbrough (2006).

Triple Sugar Iron Agar Test (TSI):

Bacterial isolates were stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was incubated at optimal temperature of 37ºC for 24 hours. The T S I slant medium was used to check for the present of the following:

GAS: if bubble is present in the media (gas positive)

H_2S: if black is present in the media (H_2S positive)

LACTOSE: If the top of the media turn from pink to yellow (lactose positive)

GLUCOSE: If the bottom of the media turns from pink to yellow (glucose positive)

Catalase Test:

This test detects the presence of catalase enzyme when present in a bacterium, it catalase the breaking down of hydrogen peroxide with the release of oxygen as bubble.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

With a wire loop, a colony was picked from the pure culture and was transferred to the center of a glass slide. 1- 2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative.

Indole Test:

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which then accumulates in the medium for indole production. Bacterial isolates were inoculated into peptone water medium contained in sterile test tubes then incubated at 37ºC for 48 hours. After the incubation period about 3 drops of kovacs indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly coloured (Bello, 2002).

Citrate Test:

The bacterial isolate was tested for their ability to utilize citrate as the sole carbon source. Simmons citrate medium was used. Bacterial isolates were inoculated into Simmons citrate medium in test tubes and incubated at 37ºC for 24 –48 hours. The culture media was observed for a colour change from green to blue. Positive showed no growth with intense blue colour, while negative test showed no growth and the colour of the medium remained green (Bello, 2002).

RESULTS AND DISCUSSION:

Results

The result shows bacterial isolated from the samples (Fried fish and Smoked fish). The bacterial isolated from samples are *Vibrio chariae, Clostridium species*, *Proteus mirabilis, Lactobacillus species*, and *Corynebacterium species.*

Table 1 Shows the morphological and biochemical characteristics of bacteria isolated. **Table 2** Shows bacterial isolates, number of occurrence and percentage of occurrence. **Table 3** Occurrence of bacteria isolates identified in different samples and heterotrophic plate count. **Table 4** Shows the mean cfu/ml count of bacteria isolates from various samples.

Table 1. Cultural, Morphological and Biochemical Characteristics

Isolates	Gram Stain	Morphologic al characteristic	Citrate	Catalase	Indole	glucose	Lactose	H_2S	Gas
Clostridium species	GPB	Rods	-	+	-	+	-	+	-
Vibrio chariae	GNB	Rods	+	+	-	+	-	-	+
Corynebacterium species	GPB	Rods	-	+	-	+	-	-	+
Proteus mirabilis	GNB	Rods	+	+	+	+	-	-	+
Lactobacillus species	GPB	Rods	-	+	-	+	+	+	+

Key = + = positive, - = Negative, GPB = Gram Positive Bacillus, GNB = Gram Negative Bacillus, GPC = Gram Positive Cocci

Table 2. Shows bacterial isolates, number of occurrence and percentage of occurrence.

Sample	Bacterial Isolates	Number of occurrences per sample	Percentage (%) of occurrence
A	*Vibrio chariae*	4	100.00
	Lactobacillus species	1	25.00
	Proteus mirabilis	2	50.00
B	*Corynebacterium species*	1	25.00
	Clostridium species		
C	*Proteus mirabilis*	3	75.00
		1	25.00
	Vibrio chariae		
D	*Corynebacterium species*	2	50.00
		2	50.00

Table 3 Occurrence of bacteria isolates identified in different samples and its heterotrophic plate count.

Sample	Bacterial Isolates	CFU/ml	CFU/ml in standard
A	*Vibrio chariae*	72	7.2×10^3
	Vibrio chariae	96	9.6×10^3
	Vibrio chariae		7.6×10^3
	Vibrio chariae	76	9.6×10^3
		96	
	Lactobacillus species		6.4×10^3
B	*Proteus mirabilis*	64	10.0×10^4
	Proteus mirabilis	100	7.6×10^3
	Corynebacterium species	76	8.8×10^3
		88	
	Clostridium species		
	Clostridium species		7.6×10^3
C	*Clostridium specie*	76	8.8×10^3
	Proteus mirabilis	88	5.6×10^3
		56	6.4×10^3
		64	
	Vibrio chariae		
	Vibrio chariae		7.2×10^3
D	*Corynebacterium species*		4.8×10^3
	Corynebacterium species	72	9.6×10^3
		48	9.2×10^3
		96	
		92	

Table.4 Shows, mean of cfu/ml count of bacteria isolates from various samples

Sample	Mean of cfu/ml
A	*85.00*
B	*82.00*
C	*71.00*
D	*77.00*

DISCUSSION:

Although Nyagambi (1986), in his study showed that artisanal women fish processors and traders purchase fresh fish of good quality to ensure better end products, this study shows that smoking and frying is usually done to the fish that were meant to be sold fresh. It is envisaged in this study that fish processors be advised to choose high quality fish products. This is because, people eat smoked and fried fish due to the flavour and texture that the fish acquires on smoking and frying and they deserve to eat products of high quality. Choice of good quality raw material will also ensure longer shelf life of the products.

Vibrio spp was the most predominant bacteria isolated from the samples analysed (table 2 and 3). This result is in line with Investigation of bacteriological quality of smoked fish by Ogwan'g, *et al.*, 2005 who isolated *Vibro spp.* from smoked fish.

This post-harvest infection of smoked fish is in line with what Dillon, *et al* (1994), studying microbiology of smoked fish in Canada found out, that the level of micro-organisms in fish reduces with smoking but increases with storage period and during transportation. It is therefore imperative that the necessary measures to arrest this situation be put in place if the smoked fish products have to remain wholesome and hygienic.

Frying dehydrates the fish flesh and kills many of the bacteria in the fish (Taofiq, 2005). The presence of

moisture in fried fish permits the growth of bacteria and mould in fish flesh during storage (Eyo, 2001).

CONCLUSION AND RECOMMENDATIONS:

Conclusion:

Finding from this study indicate the presence of bacterial contamination in samples analyzed. When consumed, they might be source of infection in humans. This suggest the need for veterinary and public health intervention through fish regulatory programs, more so fish processors should be educated on safe methods of preservation in order to prevent or minimize fungal contamination.

In view of the heavy bacterial contaminants isolated from smoked fish and fried fish in this study, fishermen and marketers should adapt better method of preservation and better smoking kilns should be provided to them at subsidized rates and oil used in frying of fish should be changed at regular intervals. More so, stored fish product should be well kept.

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