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Some Pathogenic Bacteria Associated With Fresh and Smoked Nile Perch (*Lates Niloticus)* In Luanda Market, Vihiga County (Kenya).

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Abstract: *Luanda market is located in Luanda town, Kenya. It lies 0° 0' 0" N and 34° 35' 0" E. While the Nile perch (Lates niloticus) a fresh water fish species introduced in Lake Victoria from the family latidae, order perciforme is widespread throughout much of the afro-tropic ecozone, where it is of great economic importance. In L. Victoria (Kenya), in many ways when this fish is harvested it would be contaminated by microorganisms due to poor hygienic handling or if the fish fed on food infected by microorganisms, dirty water and dirty storage. Before this study, little was known about the pathogenic bacteria flora that may be associated with smoked or fresh L. niloticus in the Luanda fish market, Luanda (Kenya), therefore this study was initiated to determine their occurrence and identities. Samples of fresh and smoked fish identified to be L. niloticus were randomly collected from Luanda market, the skin was scraped and a swab stick used to swab the fish body after which the swab was inserted into a test tube containing 9ml.of distilled water as a stock, prior to serial dilution in 5 test tubes. NA plates obtained were then incubated at 37°C for 24 hrs before pure cultures of emergent growth were subjected to isolation procedures. Identification and characterization was based on colony morphology, gram-staining technique, elevation, nature of surface, shape and opacity. Bacteria species such as Staphylococcus aureus, Streptococcus sp., Shigella sp., Escherichia coli, and Salmonella sp. were identified. Further examination of fresh fish samples indicated that they present a rich habitat for bacteria than fish smoked using fire wood. Gram staining test indicated most microbes on smoked fish were mostly gram positive and were able to retain the crystal violet color, while most bacteria found in fresh fish showed a gram negative result by failing to retain the crystal violet color. The catalase test carried indicated that a prokaryote S. aureus was catalase positive, while the rest of prokaryotes were catalase negative when they showed no bubbles. Hence it became clear from this study that fish sold in Luanda market is not fit for human consumption unless heat cooked sufficiently to eliminate all the pathogenic microorganisms' resident therein. There is need to conduct more studies on microorganisms associated with other body parts and other fish species sold in Luanda market.*

Keywords; *Isolation, characterization, pathogenic, fish, microorganisms, bacteria.*

I. Introduction

Luanda fish market is located about 25 km from Kisumu city and about 3.5 km from Maseno town. The fish Market is located at 0° 0' 0" N. and 34° 35' 0" E. within the designated municipal market of Luanda town. Fish sold is harvested mainly from L. Victoria and from fish ponds located within the larger Vihiga County and nearby Kisumu County, both counties being located in western Kenya. Fish is a low fat food, great source of protein, vitamins and minerals so much so that over the years fish farming has gained a rapid interest due to the importance since beef and even goat meat are beyond the reach of not only Luanda residents but the entire western Kenya. In Luanda market, the demand for fish greatly exceeds supply; this problem being further aggravated by the low level of domestic fish production against the increase human production. As a result of the crucial role played by fish production in meeting the protein demands there is need to promote fish farming and production to fill this shortage.

Microorganisms pathogenic to fish may be grouped broadly as; bacteria, fungi, virus etc., yet these parasites put together are responsible for about 45% loses in fish farms [1] and in lakes. In western Kenya, not much is known if pathogens resident on fish in L. Victoria or fish ponds may persist after fish harvesting and transportation to the fish market, though fish contamination may occur when the fish feeds on food infected by bacteria, fish survival in dirty waters, dirty storage conditions after harvest and unhygienic handling by humans. Before this study, little was known about the pathogenic bacteria flora that may be associated with smoked or fresh fish in Luanda fish market in Kenya, therefore this study was initiated to determine the occurrence and identity of such microorganisms.

II. Materials and Methods

Description of the area of study and sample collection.

This study was conducted in Luanda market, Luanda town; Vihiga County in western Kenya, Luanda town is located approximately 3 Km from Maseno Town, and 3.5 Km from Maseno University, where isolation and characterization was conducted. The study area is located a few kilometers from the Equator at Maseno, its geographical coordinates being 0° 0' 0" North, 34° 35' 0" East at an elevation of 1501m above sea level. The town has an estimated resident population of 14,000 people and is a significant business point lying at an intersection of major roads leading to Siaya, Busia and Majengo towns, thereby creating a lot of economic activities and a high population density. Fish samples, from which the skin resident microorganisms were isolated, were randomly collected from different selling stalls at the fish market and transported to the Botany laboratory at Maseno University, Maseno.

Serial dilution

Samples of fresh and smoked fish belonging to fish species identified as *Lates niloticus* was made using a method earlier describe [2], The part of the fish body were scraped and swab stick used to swab the fish body and inserted into the first test tube containing 9 ml of distilled water as a stock, and five other test tubes also containing 9ml of distilled water arranged serially in a test tube rack **[3]** 1ml the stock was collected using a pipette to the first test tube and from the first test tube tu the second test tube up to the fifth test tube respectively i.e. $!0^{-01}$, 10^{-02}, 10^{-03}, 10^{-04} and 10^{-05} respectively. 10^{-04} and 10^{-05} were used as the dilution factor [3]**.** 1ml was taken from each factor into a sterilized petri dish in duplicate where all plates were incubated at a temperature of 37°C for 24 hrs before isolation procedures [3].

Media preparation

NA agar used in these studies was prepared as earlier described [3].

Identification and characterization of the isolates

Emergent growth of skin resident microorganisms were later sub-cultured to obtain the pure cultures that were subjected to gram staining [3]**,** and identification conducted as earlier described [4], [5] and [6]**.**

Gram staining

A wire loop was used to make a smear of the bacteria on the slide and allowed to dry. The smear was then flooded with primary stain (crystal blue) and left for 30 seconds. This stained all the cells. The smear was then rinsed to remove the excess stain and flooded with dilute solution of iodine for 1 minute. The stained smear was rinsed again, and then 95% alcohol was then briefly added. This acted as the decolorizing agent and removed the dye-iodine complex from gram negative but not gram positive complex. A counter stain (safranin) was then applied to impart contrasting color to the now made colorless gram negative bacteria.

Catalase test

Metabolic reactions that occur in the presence of water and oxygen often result in the formation of hydrogen peroxide (H_2O_2). This compound is toxic to cells.

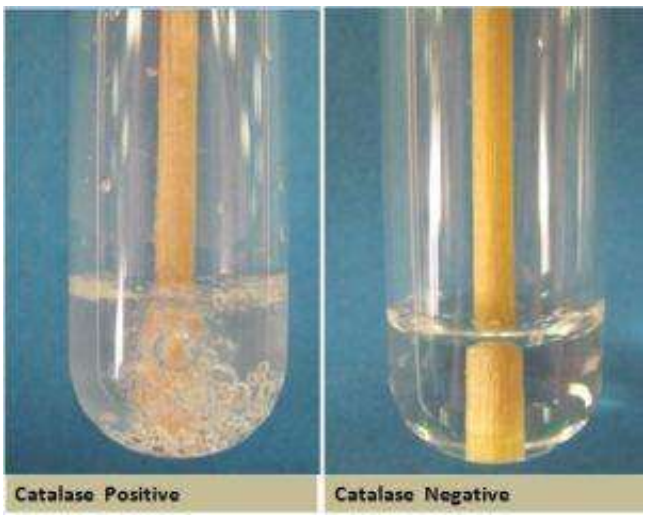

Plate 1 showing a positive and negative catalase test

Therefore, most organisms that can grow in the presence of oxygen possess catalase, an enzyme that converts hydrogen peroxide to water and oxygen.

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

5 ml of hydrogen peroxide was poured into 5 clean test tubes, and using a sterile needle, a colony from a tryptic soy agar was removed from the bacteria and mixed with hydrogen peroxide and observation made. This tested for *Staphylococcus aureus* and *Streptococcus pyogenes*. The bubble formation indicated a positive test. Absence of the bubble indicated a negative result as indicated in plate 1.

Sample preparation

Sample preparation was made using the method described by [3]. The part of the fresh fish body was scrapped and swab stick used to swab the body of the fish and inserted into the fish test tube containing 9 ml of distilled water as a stock, and five other test tubes also containing 9 ml distilled water were arranged serially in the test tube rack. 1ml of the stock was collected using a sterilized pipette to the first test tube and from the first test tube to the second test tube up to the 5^{th} test tube respectively. 1 ml was taken from each sample into sterilized petri dishes in triplicate. All the plates were incubated at a temperature of 37 degrees Celsius for 48 hours, after which sub-culturing was done after another 48 hours to obtain pure cultures and colony counts for the pure culture [3].

Bacterial colony description.

Bacterial colonies growing in NA were characterized as being opaque, transparent, translucent, surface dull, smooth and rough based on the characters exhibited by the colonies (Table 2).

III. Results and Discussions

Bacteria flora

The rich microbial flora associated with fish in this study could be as a result of the warm luxuriant environment in which the fish handled by humans before and after harvesting. In this study, fish samples that were smoked on fire wood barbecue were either displayed on dirty floor/mats/trays/open containers or untidy tables in the markets for sale. Processed fish are easily contaminated with microorganisms in nature, through handling, during processing and if the post-processing handling is not properly done under hygienic conditions. The quality of smoked products is dependent on several factors, including, the quality of the fish at the time of smoking, the preparation of the raw material, the nature of wood and the type of the smoking procedure employed by the processor. Bacterial isolates obtained from the smoked and fresh fish specimens were designated as isolate 1- 5, the characters of each isolate or colonies were described fully in table 2. Isolate 1 showed positive results with the gram staining technique, i.e. retained the color of the crystal violet, however when tested for catalase, the result was negative by showing no scanty bubbles, hence implying that the bacteria species contains no oxygen and may therefore be referred to as anaerobic (table 2). Isolate 2 showed a positive gram reaction result, and when tested for catalase test, it was then found to be positive, hence called aerobic bacteria (table 2). Isolate 3 appeared to be a rod shaped bacteria that showed a negative result with the gram reaction and negative catalase test (table 2). Isolate 4 were a rod shaped bacteria that showed negative a results with the gram reaction and showed a negative catalase result (table2). The Isolate 5 was a curved bacterium that showed a negative gram reaction test and a negative catalase test (table 2).

The result obtained in this study revealed that *Staphylococcus aureus*, *Shigella* sp., *Streptococcus species, Salmonella species, Escherichia coli* were the common pathogenic bacteria found associated with fresh and smoked fish in Luanda market. The presence of *S. aureus* was attributed to the contamination of the fish samples by man through handling and processing. [3] Though *S. aureus* has been recorded to occur, it seldom occurs as natural micro flora of fish. Its main habitat is humans and animals, and found mostly in the nose, throat and skin of healthy individuals [7]. This indicates that fresh and smoked fish with these bacteria pathogens must have been contaminated through handling during post harvest. In a similar study carried out by [8], *Staphylo*coccus sp. and *Streptococcus* sp. were all found to be associated with smoked fish. It was suspected that these organisms may have contaminated the smoked fish through human handlers, air and soil.

Table 1: Showing the number of bacterial colonies isolated from smoked and fresh fish from Luanda market

Type of fish	Dilution	**Mean**	**Std. Error**	**95% Confidence Interval** Lower bound	Upper bound
Fresh fish	Dilution1 (9 ml)	14.000	.641	12.663	15.337
	Dilution2 (9 ml)	15.667	.641	14.329	17.004
	Dilution3 (9 ml)	14.667	.641	13.329	16.004
	Dilution4 (9 ml)	9.333	.641	7.996	10.671
	*Dilution5 (9 ml)	8.333	.641	6.996	9.671
Smoked fish	Dilution1 (9 ml)	11.333	.641	9.996	12.671
	Dilution2 (9 ml)	12.333	.641	10.996	13.671
	Dilution3 (9 ml)	11.000	.641	9.996	12.337
	Dilution4 (9 ml)	7.333	.641	5.996	8.671
	Dilution5 (9 ml)	7.000	.641	5.663	8.337

The findings of [8] corroborate the findings in this study. since common bacteria such as *Staphylococcus aureus, Shigella sp.*, were also isolated in this study. the presence of these organisms in the smoked fish samples of *lates niloticus* might be due to increase in moisture content of the product during storage, and also increase in temperature that favors the growth of these organisms.

Table 2: Morphological and biochemical characterization of smoked and fresh fish sold in Luanda market

Isolate	fish	Gram Reaction	Catalase test	Bacterial growth on the N.A and colonies descriptions					Probable identification	Bacteria shapes
				color	Elevation	Surface	Shape	opacity		
1	**Fresh**	+	-	yellow	Convex	Smooth	irregular	opaque	*Streptococcus* sp.	round
2		+	+	colorless	Raised	Dull	filamentous	translucent	*S. aureus*	round
3		-	-	colorless	flat	Dull	irregular	transparent	*Salmonella* sp	rod
4		-	-	yellow	Flat	Smooth	round	opaque	*Shigella* sp.	round
5		-	-	colorless	flat	dull	irregular	translucent	*Streptococcus* sp.	curve
1	**Smoked**	+	-	colorless	Raised	dull	filamentous	translucent	*Streptococcus* sp	round
2		+	+	colorless	Flat	Smooth	irregular	translucent	*S. aureus*	round
3		-	-	colorless	Flat	Dull	filamentous	translucent	*Salmonella* sp	rod
4		-	-	Pink	Raised	dull	filamentous	translucent	*Shigella* sp.	rod
5		-	-	yellow	flat	smooth	curled	opaque	*E. coli*	spiral

During handling of fish, organisms associated with man, such as *Salmonella sp.* and *Staphylococcus aureus*, both isolated in this investigation, can grow well at 30C - 37C [9]. In a related development, [10], isolated and identified *Staphylococcus aureu, Bacillus sp., Salmonella sp.* and *Streptococcus sp.* from the skin of *Clarias gariepinus* also supports the outcome of this study. *Salmonella sp.* may be present naturally in tropical aquatic environments [10]. The microorganism isolated in this study are known constitutes of food safety problems.

It is noteworthy that sanitary condition under which fish are handled, processed and stored at the Luanda fish market need to be improved upon to reflect a good standard or good practices to minimize any infection or spread of theses microorganisms identified to be associated with *Lates Niloticus* namely; *Escherichia coli, Streptococcus* sp., *Staphylococcus aureus, Shigella* sp. and *Salmonella* sp. It is therefore correct to say that fish sold in Luanda is not safe for consumption by human unless it is well cooked to destroy all pathogenic bacteria.

IV. Conclusions

The results from this study have clearly shown that the fish obtained from Luanda market in western Kenya has a high bacteria flora. This may be due to the kind of storage subjected for the fish, and the handling procedures that are poor. Therefore strict measures should be undertaken to control level the level of bacteria flora. Fish dealers and handlers should be educated on the proper and efficient ways to store fish to avoid the contamination with the pathogens. There is need to educate the local community on hygienic aqua culture practices; such as how to rear fish that is pathogen free, and better disposal of organic components to avoid fish contamination at water bodies within the local area and inside the market premises.

Suggestions for future Research

- This study only dealt with the isolation of bacteria from the skin of fish only and not other body parts such as gills, intestine, etc. there is need for future studies to isolate all pathogens that may be associated with such parts that were not dealt with in this study.
- This study only concentrated on *L. Niloticus* species studies need to be conducted on other fish species that are sold not only in Luanda fish market but in other local markets.

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