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Isolation and Identification of Bacteria in Retailed Smoked Fish Sold in Umuahia Metropolis

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Abstract: This study was to identify the bacteria commonly involved in contamination of smoked-dried fishes; Stock fish (*Gadusmorhua*), Bonga fish (*Ethalmosafimbriota)*, Croaker (*Pseudotolithustypus*), African catfish (*Clariasgariepinus*), mackerel (*Scomberscombrus*) available in Umuahia metropolis, Eastern Nigeria. A total of 300 fish were sampled randomly from four different market location, Ndoru, Isi gate, Ahiaeke and Ubani Markets. Seventy Five fish samples were bought randomly from each of the market. Ten grams of the fish sample obtained from each of the markets was weighed aseptically and macerated in 90 ml of sterile peptone water as a stock and five other test tubes also containing 9 ml of sterile peptone water were arranged serially in the test tube rack. 1 ml. of the stock was collected using a pipette to the first test tube and from the first test tube to the second test tube up to the fifth test tube respectively. The media used were Mannitol salt agar, MacConkey agar and Salmonella Shigella agar. Mackerel fish had the highest level of contamination with bacteria followed by Croaker fish, Bonga fish, Cat fish and Stock fish respectively. Ahiaeke market had the highest number of fish contaminated with bacteria. *Staphylococcus* was one of the most prominent bacteria that the fish were contaminated with; Ahiaeke market had the highest mean of 1.81 x 10^6cfu/g, Isigate gate 1.62 x 10^6 cfu/g, Ubani 1.44 x 10^6 cfu/g and Ndoru 1.26 x10^6cfu/g, respectively.

Key words: Bacteria · Smoked · dried Fishes · Umuahia

INTRODUCTION

Fish is considered as one of the sources of proteins, vitamins and minerals and also an essential nutrients required for supplementing both infant and adult diets [1]. Fish and fish products play an important role in the diets of the populations of West African countries and it constitutes more than 60% of the total protein intake in adults, especially in rural areas. It has a relatively 10% calories content, hence its role in nutrition is recognized. In Nigeria, fish is eaten cooked, preserved or processed (smoked) and it is a delicacy that cuts across socio-economic, age, religious and educational barriers [2]. In terms of safety, fish has been implicated in several outbreaks of food-borne infections. It is a potential vehicle for food-borne infections such as cholera, listerosis, salmonellosis and many more [3-5]. Many spoilage microorganisms which are also known to be opportunistic pathogens including *Pseudomonas spp.* and *Proteus spp.* have also been associated with fish [3, 6-8]. Smoked fish constitute a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural population. These products can be kept for 2 to 4 weeks in market stalls with poor storage facilities. Smoke drying method is carried out in traditional smoking kilns of clay, cement blocks, drums or iron sheets [9]. From the point of processing to market stalls, smoked dried fishes often get contaminated with microorganisms such as bacteria, yeasts and moulds [10-12]. Various pathogenic agents that are isolated from different types of

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fish can grow and produce their toxic secondary metabolites, these are retained in fish flesh even after salting. These toxic substances cause public health hazards [13]. The smoking of fish from wood for preservation dates back to civilization [14]. Apart from giving the product desirable taste and odour smoking provides longer shelf-life through its anti-bacterial and oxidative effects, lowering of pH, as well as accelerating the drying process and acting as antagonist to spoilage agents [15, 16]. The aim of the present study is to determine and identify the bacterial pathogens contaminating smoked dried fishes in Umuahia metropolis.

MATERIALS AND METHODS

Study Area: The study area was Umuahia Metropolis.

Sample Collection: A total of 300 smoke dried fishes (Cat Fish, Stock Fish, Bonga Fish, Mackerel and Croaker Fish) will be randomly sampled and purchased from four different marketing sites located in Umuahia. These would include Ndoru, Ahaike, Isi- Gate and Ubani markets at an interval of one week for a period of four weeks. Seventy five samples of the fish species will be obtained from each market. To avoid contamination during sampling, transportation and storage, the samples will be kept in labeled polythene bags and taken immediately to Veterinary Microbiology laboratory Michael Okpara University of Agriculture, Umudike for analysis.

Sample Preparation

Preparation of Serial Dilution: Sample preparation will be made using the method described by Chessbrough [17]. Ten grams of the fish sample obtained from each of the markets will be weighed aseptically and macerated in 90 ml of sterile peptone water as a stock and five other test tubes also containing 9 ml of sterile peptone water will be arranged serially in the test tube rack. 1 ml. of the stock will be collected using a pipette to the first test tube and from the first test tube to the second test tube up to the fifth test tube respectively i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. 10^{-4} and 10^{-5} will be used as the dilution factor and 1 ml will be taken from each factor into a sterilized petri dish in duplicate. All plates will be incubated at a temperature of 37°C for 24 hrs, before colony counting and isolation procedures.

Media: The media used includes: Mannitol salt agar (MSA) was used for *Staphylococcus aureus*; MacConkey Agar (MCA) was used to enhance the growth of Gram-negative organisms and as a multipurpose agar; Salmonella Shiggella agar (SSA) was used for culturing Salmonella and Shigella species. All media for isolation of the organisms was prepared aseptically according to manufacturer s specification.

Inoculation of Media Plates: Pour-plate method was adopted for inoculation of all media plates. The plates were then be subjected to 24 hours incubation at 37°C after which all plates were read. Each discrete colony observed was picked and streaked on fresh media plates to obtain pure cultures. All plates and test tubes used were properly labeled according to dilution, agar used and Market from which the sample was obtained. Bacterial growths from the plates were subjected to Gram staining test and biochemical test. (Coagulase, Catalase, Citrate utilization test and Indole etc.) tests, to confirm their identity.

Bacteria Colony Count: Bacteria colonies was counted using colony counter machine. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1 ml of the original sample and plating was done in duplicate for each dilution. An average count was taken to obtain the total count. The colony counts of duplicate plates was calculated by Average count × dilution factor.

RESULTS

A total of 300 of fish were sampled randomly from four (4) different market locations Ndoru, Isi Gate, Ahiaeke and Ubani markets, (75) samples were bought randomly from each of the market. Table 1 shows that mackerel fish had the highest number of fish with bacterial contamination followed by croaker fish, Bonga fish, cat fish and stock fish respectively. Table 2 shows that Ahiaeke market had the highest percentage of fish contaminated with bacteria (80%) followed by Ndoru, Isi-gate and Ubani Market respectively. Table 3 shows that Ndoru market had 38.8% of the fish samples been contaminated with *Staphylococcus* spp, 33.3% contaminated with *Micrococcus* and 27.7% contaminated with *Bacillus* spp. Ahiaeke market had 35% of the fish samples contaminated with *Bacillus* and *Micrococcus* and 30% with *Staphylococcus* spp. Isi-gate market had 53.3% of the fish contaminated with Staphylococcus with 26.6% contaminated with *Micrococcus* and 20% contaminated with Bacillus. Ubani Market had 38.4% of the fish sample contaminated with Staphylococcus spp.

Table 1: Prevalence of bacterial contamination based on type of fish

S/N	Type of fish	No. Sampled	No positive (%)
1	Cat Fish	60	36(60)
2	Stock Fish	60	30 (50)
3	Bonga Fish	60	39 (65)
4	Croaker Fish	60	45 (75)
5	Mackerel Fish	60	48 (80)
	Total	300	

Table 2: Prevalence of bacterial contamination based on market location

S/N	Market locations	No. Sampled	No positive%
1	Ndoru	75	54 (72%)
2	Ahiaeke	75	60 (80%)
3	Isi Gate	75	45 (60%)
4	Ubani	75	39 (52%)
	Total	300	

Table 3: Percentage occurrence of bacteria in the fish samples

S/N	Market Location	Isolate	Number	Percentage (%)
1	Ndoru	Bacillus spp.	15	27.7
		Staphylococcus spp.	21	38.8
		Micrococcus spp.	18	33.3
2	Ahiake	Bacillus spp.	21	35
		Staphylococcus spp.	18	30
		Micrococcus spp.	21	35
3	Isi gate	Bacillus spp.	9	20
		Staphylococcus spp.	24	53.3
		Micrococcus spp.	12	26.6
4	Ubani	Bacillus spp.	12	30.7
		Staphylococcus spp.	15	38.4
		Micrococcus spp.	12	30.7

Table 4: Mean Bacteria Count of the Smoked Fish Samples

Market Location	Total no of samples	Mean count values (cfu/g)
Ndoru	75	1.28×10^6
Ahiake	75	1.81×10^6
Isi –gate	75	1.62×10^6
Ubani	75	1.44×10^6

Table 5:Cultural and Morphological Characteristics of bacteria in the Smoked fish Samples

Suspected Isolates	Morphology	Microscopic appearance
Bacillus spp.	Creamy white, raised with rough edges	Gram positive bacilli
Staphylococcus spp.	Creamy, slightly raised with smooth edges	Gram positive cocci
Micrococcus spp.	Creamy, slightly raised with smooth edges	Gram positive cocci

Table 6: Biochemical test of bacterial isolated from the fish samples

Organism	Catalase	Coaguluse	Urease	Suc	H2S	Citrate	Vp	MR	Indole	Maltose	Mannitol	Lactose	Glucose	Oxidase	Motility	Gram
Bacillus	+	NA	-	+	-	-	+	-	-	+	-	+	-	-	+	+
Staphylococcus	+	+	-	+	NA	NA	+	-	NA	+	+	+	+	-	NA	+
Micrococus	+	-	NA	+	NA	NA	-	+	NA	+	+	+	+	+	-	+

NA=Not applicable. + Positive. -Negative

with 30.7% contamination with Bacillus spp. and *Micrococcus* spp. Table 4 shows the mean bacteria count for the various market location. Ahiaeke market had the highest mean of 1.81×10^6cfu/g, Isigate gate with 1.62×10^6 cfu/g, Ubani 1.44×10^6cfu/g and Ndoru 1.26×10^6cfu/g respectively. Table 5 shows the cultural and morphological characteristics of bacterial isolates and Table 6 shows the biochemical test used in characterization of the bacterial Isolates.

DISCUSSION

This study shows that pathogenic bacteria are present in smoked fish sold in, Ndoru, Isigate, Ahiaeke and Ndoru Market. The bacteria organisms isolated from the fish include *Bacillus* spp., *Staphylococcus* spp. and *Micrococcu*s spp. This result agrees with that of Brown; Umoh and Odoba, Okonta and Ekelemu [18-20]. *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 [20] and Okonko *et al*. [21] 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*. [22] 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*. [23]. *Bacillus* and *Micrococcus* were also found to be present in the smoked fish samples which agrees with a similar study carried out by Moshood and Tengku Haziyamin [24], *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 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 [25, 26]. 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 as showed by Mepba *et al*. [27]. The mean bacteria count in the smoked fishes sampled in this study was between 1.26 x10^6cfu/g to 1.81 x 10^6 cfu/g.

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.

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.

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