

Mycological Evaluation of Smoked-dried Fish Sold at Maiduguri Metropolis, Nigeria: Preliminary Findings and Potential Health Implications

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Abstract

Background

Smoked-dried fish are largely consumed as source of nutrient by man. It has been established that fish food can act as vehicle for transmission of some mycological pathogens especially in immunocompromised individuals.

Materials and Methods

Between 7th October 2011 and 5th January 2012, a total of 100 different species of smoke-dried fish comprising 20 each of Cat fish (*Arius hendeloti*), Tilapia (*Oreochromis niloticus*), Stock fish (*Gadus morhua*), Mud fish (*Neoxhanna galaxiidae*) and Bonga fish (*Enthymosa fimbriata*) were purchased at Baga motor park in maiduguri metropolis, Nigeria. They were processed and investigated for possible fungal contamination based on culture isolation using Sabourand dextrose agar (SDA) and microscopy.

Results

Organisms isolated and identified in pure culture were *Mucor* spp. (36%), *Aspergillus niger* (35%), *Aspergillus fumigatus* (6%), *Candida tropicalis* (3%), *Candida stellatoidea* (2%), *Microsporum audunii* (2%), *Penicillium* spp. (2%) and *Trichophyton rubrum* (1%) while *Mucor* spp. and *Aspergillus niger* (4%); *Mucor* spp and *Candida tropicalis* (3%); *Aspergillus fumigatus* and *Mucor* spp. (1%); *Aspergillus niger*, *Candida* spp. and *Mucor* spp. (1%) were isolated in mixed culture. The mean colony count of pure fungal colonies ranged between 1.3×10^4 - 8.5×10^6 CFU/g. of fish extract while that of mixed fungal colonies were between 2.0×10^4 - 5.1×10^4 CFU/g.

Conclusion

Findings from this study indicate presence of fungal contamination in test fish. When consumed, they might be source of human infections. This suggests the need for veterinary and public health interventions through fish regulatory programs, more so fish processors should be educated on safe methods of preservation in order to prevent or minimize fungal contamination.

Keywords: Fish contamination; Mycotic agents; Health implications; Fish preservation

Introduction

Fish is an aquatic vertebrate with fins, gills and skin with glandular secretion that decreases friction. A typical fish is torpedo-shaped and usually limbless, with a head containing a brain or sensory organ and muscular tail. Most fish have scales and are poikilothermic [1]. Fish are extremely perishable food. For example, most fish become inedible within 12 hours at tropical temperatures. Spoilage begins as soon as the fish dies and processing therefore should be promptly done to prevent the growth of microorganisms. Fish is a low acid food and therefore susceptible to growth of food poisoning pathogens making this another reason why they should be processed quickly [2].

Preservation of fish by smoking is carried out after they are catch from water bodies, thereafter smoked fish may be eaten without further cooking. From the processing units to market centres, smoked-dried fish are often contaminated with microorganisms such as bacteria, yeasts and moulds [3,4,5]. Numerous pathogenic agents isolated from different types of fish are able to grow and produce their toxic secondary metabolites, which are retained in fish flesh even after salting and storage periods. These toxic substances caused serious systemic dysfunctions and public health hazards [6].

The smoking of fish from smouldering wood for preservation dates back to civilization [7]. It was also noted that apart from giving the product desirable taste and odour, smoking provides longer shelf-life through its anti-bacterial and oxidative effects, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage agents [8,9].

Fish fungal contamination is a serious problem that could affect fish farmers and consumers in Africa. These contaminations may be primarily by fungi (the initial agents) or as a secondary invasion of tissues already damaged by viral, bacterial or mechanical agents [1,9]. Several foodborne moulds and possibly yeasts may also be hazardous to human health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking [10]. Even though the contaminant organism may not survive food preparation, the preformed toxin may still be present. Certain moulds and yeasts may also elicit allergic reactions or may cause infection in Humans. Although most foodborne fungi are not infectious, some species can cause infection in immunocompromised populations, such as children, the elderly and debilitated individuals, Diabetic patients, HIV-infected individuals and persons receiving long-term antibiotic therapy [10]. These pose serious health implications especially when these microorganisms and /or toxins find their way into the digestive [10]. Several cases of human gastroenteritis, severe diarrhoea and food poisoning outbreaks were recorded after smoking fish consumption [10].

Saprolegnia is the most important fungal contaminant of fish, although a number of other species have been implicated. Martin [11] stated that bacteria (*Staphylococcus aureus*), yeasts (*Saccharomyces cerevisiae*) and moulds (*Penicillium* and *Aspergillus*) were the commonest microorganisms associated with smoked fish. The threshold colony forming unit (CFU) responsible for clinical diseases depends on immunity of individuals and largely vary with the type of fungus ingested. In most cases, 5×10^4 CFU/g of total source (e.g., food, water and air) are responsible for most clinical diseases [12].

Since it has been established that fish can act as vehicle for transmission of some mycological pathogens especially in immunocompromised subjects, this mycological evaluation of five species of smoked-dried fish sold at Maiduguri metropolis was conducted in order to detect the presence and determine the fungal load on smoked-dried fish sold at Maiduguri metropolis, Nigeria.

Materials and Methods

Sample collection

Between 7th October 2011 and 5th January 2012, a total of 100 different species of smoke-dried fish comprising 20 each of Cat fish (*Arius hendeloti*), Tilapia (*Oreochromis niloticus*), Stock fish (*Gadus morhua*), Mud fish (*Neoxhanna galaxiidae*) and Bonga fish (*Enthalmosa fimbriota*) were purchased at Baga motor park in maiduguri metropolis, Nigeria. The smoked dried fish were collected in sterile polythene bags and transported to the laboratory for processing and investigations. The samples were first examined macroscopically for external fungal growth. Each species of fish were identified by means of labels placed on fish by the fish-seller, more so, confirmation was done by technical assistance rendered by postgraduate students of zoology and fish biology at University of Maiduguri, Nigeria.

Protocol for smoke drying fish in Maiduguri metropolis

Fresh fish was washed clean and placed in containers or coolers and covered with ice blocks. The fish was dipped into brine (a mixture of ¼ cup of salt to 1 quarter of cold water) for 20 s. The fish were placed in a smoke house hanger or racks wiped with vegetable oil and allowed to

dry for about 1 hour. A shiny-like layer formed on the fish surface seals the surface and prevent loss of natural juices during smoking. During smoking in the smoke house, all combustible materials around and under the smoking area were cleared. The fish were laid on the wire gauze in the oven and fired from below and were smoked for about 4-5 days. when smoking was completed, the fish were removed and allowed to cool. Later they were stored in containers where they are protected from dust and insects.

Sample processing and mycological investigations

All fish samples collected for each fish species were surface sterilized with 70% ethanol and rinsed with three changes of sterile distilled water. A 10 g tissue portion of each fish species was cut from the abdominal region with a sterile forceps, grinded aseptically in a porcelain mortar and mixed in 90 mL of sterile peptone water. From this mixture, 20 microliter was plated in duplicates on Sabourand Dextrose Agar (SDA) supplemented with streptomycin to inhibit bacterial growth. The fish samples were analyzed in batches; no more than twenty (20) fish were bought and analyzed in a day. One of the inoculated plates for each sample was incubated at room temperature (25°C) while the other was incubated at 37°C for 2-3 weeks. The cultures were examined for growth at regular intervals.

The mean number of all fungal colonies appearing in the two plates was taken as the average number of colonies per plate for each fish species. The mean colony count of each fungal isolate was determined by adding up the mean number of colonies from individual paired plate and divided by the number of fish cultures identified with the particular fungi. Each different appearing culture was transferred with a sterile needle to a slide, teased apart and stained with lactophenol cotton blue and examined microscopically. All observed colonies were subcultured to obtain pure cultures which were subsequently isolated and identified using morphological characteristics, spore formation, the production of fruiting bodies and biochemical reactions. More so, colony identification was made easier by the use of mycology atlases [13,14].

Procedure for fungal identification

Lactophenol cotton blue staining (Needle mounts preparation): A drop of Lactophenol cotton blue stain was placed on a clean grease-free glass slide. A small fragment of cottony, woolly or powdery colony was picked at mid-point of culture using a sterile straight wire and teased in the stain until a homogenous blue mixture of stain and culture was obtained. A clean cover slip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation was examined using 10X and 40X objectives of the microscope respectively.

Microconidia, macroconidia, chlamydiospores and hyphae which appeared spiral, pertinate, and antler-like structures were investigated. Features seen in stained slide were compared with established characteristic fungal features using mycology atlases [13,14].

Germ tube test: This test was used for the identification of *Candida albicans* from other species. About 0.5 mL of sterile human serum was dispensed into a test tube, using a sterile wire loop; the serum was lightly inoculated with the test organism. The test tube was then incubated at 37°C for 2-3 hours, after which a drop of the serum yeast culture was transferred to a clean, grease free glass slide and covered with a cover slip. It was then examined microscopically using 10X and 40X objectives with the diaphragm closed sufficiently to give a good

contrast. Sprouting or tube-like outgrowth from the cells indicates that the organism is *Candida albicans* [13,14].

Estimation of fungi mean colony count (CFU/gram of fish)

90 mL of sterile peptone water was homogenized 10 g of the each fish sample to make 1/10 dilution, while 9 mL of sterile water was put in 6 sterile test tubes and serial dilution of the homogenized samples was made to the 6th tube. 1ml was discarded from the 5th tube and 1ml of the dilution was spread on well dried Sabouraud Dextrose agar. It was allowed to dry and incubated at room temperature for 5 days. Fungi colonies seen were estimated accordingly and mean value of various estimated counts in the two plates calculated [15].

Results

A total of 100 different species of smoke-dried fish comprising 20 each of Cat fish (*Arius hendeloti*), Tilapia (*Oreochromis niloticus*), Stock fish (*Gadus morhua*), Mud fish (*Neoxhanna galaxiidae*), and Bonga fish (*Enthalmosa fimbriota*) were purchased at Maiduguri metropolis, Nigeria for the detection of the possible presence of contaminating mycotic agents based on cultural appearance and microscopy (Table 1).

S. No.	Common name	Scientific name	Number of samples
1	Tilapia	<i>Oreochromis niloticus</i>	20
2	Stock fish	<i>Gadus morhua</i>	20
3	Bonga fish	<i>Enthalmosa fimbriota</i>	20
4	Cat fish	<i>Arius hendeloti</i>	20
5	Mud fish	<i>Neoxhanna galaxiidae</i>	20

Table 1: Species of fish investigated for mycological contaminants.

Fish Species						
Organism	Tilapia	Stock	Bonga	Cat	Mud	Total
<i>Aspergillus niger</i>	8	6	7	8	6	35
<i>Mucor</i> spp.	8	6	7	7	8	36
<i>Aspergillus fumigatus</i>	1	1	2	2	0	6
<i>Candida tropicalis</i>	1	0	1	0	1	3
<i>Candida stellatoidea</i>	0	0	1	0	1	2
<i>Microsporium audunii</i>	0	1	0	1	0	2
<i>Trichophyton rubrum</i>	0	1	0	0	0	1
	1	1	2	0	0	4
<i>Candida albicans</i>	0	1	0	1	0	2

<i>Mucor</i> spp. and <i>Aspergillus niger</i>	1	3	0	0	0	4
<i>Aspergillus fumigatus</i> and <i>Mucor</i> spp.	0	0	0	1	0	1
<i>Mucor</i> spp. and <i>Candida tropicalis</i>	0	0	0	1	2	3
<i>Aspergillus niger</i> , <i>Mucor</i> spp. and <i>Candida tropicalis</i>	0	0	0	0	1	1
Total	20	20	20	20	20	100

Table 2: Mycological contaminations of fish according to species.

Organism	No. of isolates	CFU/gram of fish
<i>Aspergillus niger</i>	35 (35%)	8.5×10^6
<i>Mucor</i> spp.	36 (36%)	1.3×10^6
<i>Aspergillus fumigatus</i>	6 (6%)	4.5×10^6
<i>Candida tropicalis</i>	3 (3%)	5.1×10^4
<i>Candida stellatoidea</i>	2 (2%)	4.0×10^4
<i>Trichophyton rubrum</i>	1 (1%)	6.3×10^6
<i>Microsporium audunii</i>	2 (2.0%)	5.5×10^4
<i>Penicillium</i> spp.	4 (4%)	1.3×10^4
<i>Candida albicans</i>	2 (2%)	4.5×10^6
<i>Mucor</i> spp. and <i>Aspergillus niger</i> (mixed growth)	4 (4%)	2.0×10^4
<i>Aspergillus fumigatus</i> and <i>Mucor</i> spp. (mixed growth)	1 (1%)	3.5×10^4
<i>Mucor</i> spp. and <i>Candida tropicalis</i> (mixed growth)	3 (3%)	5.1×10^4
<i>Aspergillus niger</i> , <i>Mucor</i> spp. and <i>Candida tropicalis</i>	1 (1%)	4.2×10^4

Table 3: Number of isolates (%) and their corresponding mean fungal colony count.

Investigations from this study revealed that *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Mucor* spp., *Penicillium* spp., *Trichophyton rubrum*, *Microsporium audunii*, *Candida tropicalis*, *Candida stellatoidea* were found to be associated with smoked dried fish sold at Maiduguri metropolis. *Mucor* spp. and *Aspergillus niger* were the dominant fungi recorded in association with the smoked dried fish contamination in this study (Tables 2 and 3). *Penicillium* spp., *Aspergillus fumigatus*, *Candida stellatoidea*, *Candida tropicalis* and *Candida albicans* occurred less frequently.

Discussion

We were able to isolate fungal agents from our test samples. This is not surprising because according to Akande and Tobor [16], freshly

caught fish covered with damp sacks mixed with wet grass or water weeds to reduce the temperature make fish prone to contamination with microorganisms such as bacteria and fungi. This indicates that spoilage of fish starts from the aquatic ecosystem.

Handling of fish could also engender microbial contamination especially in artisanal fishery due to unhygienic methods of reducing temperature. During the smoke-drying period, smoking kilns used in artisanal fishery and the overloading of the fish in the trays leads to improper processing which in turn encourages fungal attack [9,16]. During storage of smoked-dried fish product, good storage practices are not observed by most wholesalers such as improper ventilation and easy access to pests into the storage environment.

The environment where fish are displayed in the market are usually unhygienic and this could constitute another avenue for microbial contamination. Very often, retailers display the smoked dried fish samples in open trays beside refuse heaps, this encourages fungal attack through air droplets [16].

It is important to state that majority of the fungal agents isolated were of medical significance. The occurrence of *Aspergillus* spp., *Penicillium* spp. and *Candida* spp. could lead to mycotoxin elaboration and when consumed, they induce gastrointestinal and metabolic disturbances [11].

The source of fungal contaminations can also be a result of consumption of fungal contaminated feed present in the pond. Moreover, the decomposition of these feed also add to increase in fish contamination. There might be certain other conditions in the pond which favour the possibility of fungal contamination; particularly, poor pond management, injured fish or fish having other forms of diseases. Fungal organisms pose wide range of contamination in fish farming mainly due to mismanagement of ponds [17].

Fungi isolated from this present study are in consonance with findings by others authors [18-20] however, Refai et al. [20] reported that *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. are normal mycoflora present in most fish. Notwithstanding, many fungal genera have virulence factor which cause toxin elaboration under favourable predisposing environment. Ecology is also an important factor which influences the diversity of fungus genera on fish and their eggs [21]. According to Pailwal et al. [22], diversity of water molds depends upon the interaction of physicochemical factors.

The fungi isolated from our smoked-dried fish were somewhat specific in that while *Aspergillus* spp. and *Mucor* spp. were observed in all the five fish species, *Candida tropicalis* and *Penicillium* spp. occurred in tilapia while stock fish harbours most of different species of fungal isolates. Conversely, Bonga fish harbored least fungal agents. This fungal specificity may be due to the differences in the biochemical composition of these fish species to which different moulds and yeast react differently [19,23,24].

Conclusion

In view of the heavy fungal contaminants isolated from smoked-dried fish in this study, fishermen and marketers should adapt better method of preservation and better smoking methods should be provided for them at subsidized rates. More so, stored fish product should be well kept. It is important that mycological examination of fish be carried out, especially by environmental/public health workers and regulatory bodies like Nigerian National Food and Drug Administration and Control (NAFDAC) at regular intervals and the

health implications of fungal agents and their mycotoxins be emphasized to fish handlers and consumers.

On a general note, health education/enlightenment will be of great significance to fishermen, fish handlers, sellers and buyers that good processing and availability of storage facilities are crucial to minimize general microbial contamination.

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Conflict of interest

Authors declare that there is no conflict of interest associated with this manuscript.

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