NIGERIAN JOURNAL OF BIOTECHNOLOGY

Antibiotic Sensitivity Pattern of Microorganisms Isolated from Smoked and Frozen Fishes Sold in Benin and Warri Metropolis

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Abstract
Antibiotic sensitivity pattern of microorganisms isolated from smoked and frozen fishes sold in Benin and Warri metropolis were investigated. Adopting microbiological standard techniques, the results of the bacterial counts and fungal counts ranged from 5.4×10^6 (Ekpan market) to 25.1×10^6 (Ekpan market) and 1.1×10^5 (Ekpan and New Benin market) to 2.9×10^5(Effurun market). Six bacterial species namely; Staphylococcus spp., Escherichia coli, Bacillus spp., Pseudomonas sp., Corynebacterium sp., Micrococcus sp. and four fungal species namely; Aspergillus spp., Fusarium sp., Mucor sp. and Penicillium spp. were identified. Majority of the bacteria were isolated from fish samples from Benin City Markets (51.1%, n = 23) while (48.9%, n = 22) were obtained from Warri Markets. Bacillus spp. were the most predominant (35.6 %) and Micrococcus sp. was the least predominant among the bacterial isolates (2.2%). Aspergillus sp. was recorded the most predominant (37.1%) while Penicillium sp. (14.3%) was the least predominant among the isolated fungi. The isolated bacteria (Escherichia coli, Bacillus sp., Staphylococcus sp., Pseudomonas sp., Corynebacterium sp. and Micrococcus sp.) were sensitive to ciprofloxacin and showed resistance to zinnacef and rocephin. These findings show that fish samples from different markets harbour varied levels of potentially pathogenic organisms which could pose health threat to consumers. Proper handling, processing and storage methods should be put in place to reduce potential health risk.

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Introduction

Fishes are known to be highly nutritious and excellent sources of animal protein, which is consumed by a larger percentage of the world's population because of its availability and palatability (Foran et al., 2005; Sutharshiny and Sivashanthini, 2011). Marketing of fish in Nigeria is mostly carried out by local fish sellers (Okoro et al., 2010), hence it's harvesting, handling processes and distribution provides livelihood for millions of people. Fish flesh contains significantly low lipid and high water compared to that of beef or chicken (Nestel, 2000) and rich in vitamins and minerals for both young and old consumers (Edem, 2009; Koffi-Nevny et al., 2011).

Contamination of fish and other fish products by microbes have been a serious threat to human health. The improper handling and storage processes of fish pose serious effects thereby limiting the shelf life of the products (Sallam, 2007). The quality of fish products degrades due to complex processes in which physical, chemical and micro-biological forms are implicated (Sallam, 2007). Doyle (2007) had reported that storage and processing conditions affect microbial growth. Thus the need for proper handling, storage facilities and the knowledge of spoilage pattern at tropical fishes and their shelf life under ambient condition is very imperative (Okoro et al., 2010).

The smoking of fish from smoldering wood for its preservation dates back to civilization (Olokor, 2007). It's also noted that apart from giving the product a desirable taste and odour, smoking provides longer shelf life through its anti-bacterial and oxidative effect, lowering of pH, imparting, desirable coloration

as well as accelerating the drying process and acting as antagonist to spoilage agents (Sengor et al., 2004; Eyo, 2001; Homer, 1997). Martin (1994) stated that organisms such as Staphylococcus aureus, Saccharomyces cerevisiae, Penicillium and Aspergillus were the commonest micro-organisms associated with smoked fish. Freezing is a widely used method for the preservation of fish which involve the use of refrigeration, cold rooms and icing etc. Freezing generally slows down the deterioration of fish at low storage temperature (Sivertsvik et al., 2002).

The aim of this study was to investigate the microbiological qualities and antibiotic sensitivity pattern of the isolated bacteria from smoked and frozen sold in markets in Benin-City and Warri.

Materials and methods

Collection of samples: Smoked and frozen fish samples were obtained from two different Markets in Warri (Ekpan and Effurun Markets) and Benin City (Uselu and New-Benin Markets). The fish samples were collected in labelled aseptic containers and transported to the laboratory for analysis.

Enumeration and isolation of microbial isolates: Ten grams of fish sample was weighed into 90 ml of sterilized peptone water for serial dilutions processes (10^{-4} and 10^{-5}) were prepared and used for serial dilution. Appropriate dilutions were plated in duplicates on sterile nutrient agar and potato dextrose agar for total heterotrophic bacterial and fungal counts respectively. The PDA agar

plates were made selective for fungal growth by the addition of 1 ml of 250 mg/ml chloramphenicol solution prior to pouring of the molten medium. The agar plates were incubated aerobically at 30 ^{o}C for 48 hours and 5 days at room temperature (28°C ± 5°C) for total heterotrophic bacterial and total fungal counts respectively. After incubation, counts obtained were recorded and expressed as the colony forming unit per gram (cfu/g) of the original sample (Harley and Prescott, 2002).The various isolates were further identified and characterized using the methods of Barnett and Hunter, 1975; Cheesbrough, 2006.

Antibiotic Sensitivity Testing: Antibiotics sensitivity patterns of the bacterial isolates were determined using the disc diffusion method (Cheesbough, 2003). The discs were placed on Muller-Hinton agar plates that were seeded with the broth culture of the test organisms. The plates were inverted and left on the work bench for 30 min to allow for diffusion of antibiotics into the agar, this was followed by incubation at 37^{0}C for 48hr, after which zones of inhibition were examined and interpreted using standard charts (NCCLS, 2003).

Results

Table 1 shows the total bacterial and total fungal counts of fish samples from Markets in Warri and Benin City. The bacterial count ranged from 5.4 x 10^{6} (Ekpan market) to 25.1 x 10^{6} (Ekpan market) while the fugal counts ranged from 1.1 x 10^{5} (Ekpan and New Benin market) to 2.9 x 10^{5}(Effurun market).

Table 1: Total viable count for bacterial and fungal isolate

Markets	Smoked Fish		Frozen Fish	
	Total Bacterial Counts Cfu/g	Total Fungal counts Cfu/g	Total Bacterial Counts Cfu/g	Total Fungal counts Cfu/g
EKM	5.4 x 10^6	2.0 x 10^5	23.2 x 10^6	2.5 x 10^5
EKM	7.0 x 10^6	1.1 x 10^5	25.1 x 10^6	1.2 x 10^5
EKM	9.1 x 10^6	3.0 x 10^5	19.0 x 10^6	1.8 x 10^5
EFM	10.4 x 10^6	4.4 x 10^5	18.2 x 10^6	2.9 x 10^5
EFM	9.2 x 10^6	2.3 x 10^5	17.2 x 10^6	1.8 x 10^5
EFM	8.4 x 10^6	1.7 x 10^5	21.6 x 10^6	1.3 x 10^5
USM	7.0 x 10^6	2.3 x 10^5	16.8 x 10^6	1.8 x 10^5
USM	5.6 x 10^6	1.40 x 10^5	9.8 x 10^6	1.3 x 10^5
USM	8.1 x 10^6	1.70 x 10^5	8.8 x 10^6	2.1 x 10^5
NBM	4.4 x 10^6	1.3 x 10^5	11.6 x 10^6	1.6 x 10^5
NBM	6.4 x 10^6	1.1 x 10^5	20.8 x 10^6	1.4 x 10^5
NBM	7.2 x 10^6	1.5 x 10^5	8.0 x 10^6	1.7 x 10^5

KEY: EKM = Ekpan market, EFM = Effurun market,
USM = Uselu market, NBM = New Benin market

Table 2 shows the percentage frequency of occurrence of bacterial isolates obtained from the fish samples bought from markets in Warri and Benin City respectively. The result shows that Bacillus sp. was the most predominant (35.6 %) followed by Staphylococcus (31.1%), Escherichia coli (17.8%), Pseudomonas sp. (8.9 %), Corynebacterium sp. (4.4 %) and Micrococcus sp. was the least predominant among the bacterial isolates (2.2%). Majority of the bacteria were isolated from fish samples from Benin City Markets (51.1%, n = 23) while (48.9%, n = 22) were obtained from Warri Markets.

Table 2: Percentage frequency of occurrence of bacterial isolates

Isolate	No (%)	Market Locations (%)	
		Warri (%)	Benin (%)
Bacillus spp.	16 (35.6)	8 (36.4)	8 (34.8)
Staphylococcus spp.	14 (31.1)	6 (27.3)	8 (34.8)
E. coli	8 (17.8)	4 (18.2)	4 (17.4)
Pseudomonas sp.	4 (8.9)	3 (13.6)	1 (4.4)
Corynebacterium sp.	2 (4.4)	1 (4.5)	1 (4.4)
Micrococcus sp.	1 (2.2)	0 (0.0)	1 (4.4)
Total	45 (100)	22 (48.9)	23 (51.1)

Table 3 shows the percentage frequency occurrence of fungal isolates from this study. Aspergillus sp. was recorded the most predominant (37.1%) while Penicillium sp. (14.3%) was the last predominant among the isolated fungi. The results of the antibiotic sensitivity pattern of bacterial isolates are shown in Table 4. The inhibitory zones of antibiotic resistance and sensitivity (mm) revealed variable antibiogram patterns of the tested isolates. The isolated bacteria (Escherichia coli, Bacillus sp., Staphylococcus sp., Pseudomonas sp., Corynebacterium sp. and Micrococcus sp.) in this study were sensitive to ciprofloxacin and showed resistance to zinnacef and rocephin.

Discussion

The presence of microorganisms in fishes is not out of place, when viewed as a variable substrate for the proliferation of these organisms. However, when considered from the public health stand point, growth and presence of microorganisms in commercially sold smoke and frozen fishes is unacceptable. The results of this study demonstrated the significant presence of saprophytic and pathogenic microbial flora in commercially sold smoked and frozen fishes from major Markets in Benin City and Warri.

The isolated microbial isolates included six bacterial and four fungal isolates. They were Staphylococcus spp., Bacillus spp., Pseudomonas sp., Micrococcus sp., Escherichia coli, Corynebacterium sp., Aspergillus spp., Fusarium spp., Penicillium spp., and Mucor sp.

The presence and high number of these microbial genera could be explained by the fact that these organisms can withstand the adverse processing conditions. These bacterial and fungal isolates have earlier been implicated as major pathogens associated with fish spoilage (Gram and Huss, 2001). According to Adebayo-Tayo et al. (2011), contamination of fish may occur from food handlers and retailers who sell these items to the public for consumption. Bacillus sp. and Aspergillus sp. had the highest frequency of occurrence among the various bacterial and fungal isolates which may have been occasioned by poor handling or cross contamination (Gupta et al., 2006). Also, organisms like Bacillus and fungal genera such as Aspergillus and Penicillium spp. were reported to survive for a long time in a dormant state due to the spore forming state.

The presence of indicator organisms like Escherichia coli might be as a result of possible contamination during sales and unhygienic handling of fish products. The isolation of Pseudomonas sp. from the fish samples is of high importance because it plays a considerable role as a potential pathogenic bacteria for human, also as an indicator of food quality and as a good spoilage index (Jeyesekaran et al., 2006; Yagoub, 2009), and it was prevalent in the fish sampled from Warri market.

Table 3: Percentage frequency of occurrence of fungal isolates

| Isolate | No (%) | Market Locations (%) | |
		Warri (%)	Benin (%)
Aspergillus spp.	13 (37.1)	7 (38.9)	6 (35.3)
Fusarium spp.	10 (26.6)	6 (33.3)	4 (23.5)
Mucor sp.	7 (20.0)	2 (11.1)	5 (29.4)
Penicillium spp.	5 (14.3)	3 (16.7)	2 (11.8)
Total	35 (100)	18 (51.4)	17 (48.6)

Table 4: Antibiotics Sensitivity Test Result

| Antibiotic | Test Isolate | | | | | |
	Escherichia coli	Bacillus sp.	Staphylococcus sp.	Pseudomonas sp.	Corynebacterium sp.	Micrococcus sp.
PEF	12mm	Nil	Nil	14mm	17mm	Nil
CN	12mm	Nil	Nil	7mm	15mm	Nil
APX	8mm	Nil	Nil	Nil	Nil	11mm
Z	Nil	Nil	Nil	Nil	Nil	10mm
AM	10mm	Nil	Nil	Nil	Nil	Nil
R	Nil	Nil	Nil	Nil	Nil	Nil
CPX	9mm	13mm	16mm	9mm	11mm	9mm
S	11mm	Nil	Nil	Nil	Nil	10mm
SXT	10mm	Nil	Nil	Nil	Nil	Nil
E	Nil	Nil	Nil	12mm	16mm	7mm

KEY: PEF = Perfloxacin, CN = Gentamycin, APX = Ampiclox, Z = Zinnacef, AM = Amoxacilin, R = Rocephin,
CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin, Nil = no zone of inhibition

Staphylococcus sp. produce a variety of extra cellular enzymes and toxins that have been found to be responsible for food poisoning and can rapidly develop resistance to many anti-microbial agents and pose a therapeutic problem. Aspergillus sp. and related mould generally grow faster and are more resistance to high temperature and low water activities and tends to dominate spoilage in warmer climate (Doyle, 2007).

Ogunshe and Olabode (2009) reported antibiotic resistance among bacterial species isolated from the fish (Clarias gariepinus). According to Malik and Ahmad (1994), high levels of bacterial resistance to antibiotics are indications of abuse and misuse of antibiotics in the society. This involves situation where individuals purchase and consume antibiotics with out and medical advice. Bacterial groups co-habiting a common environment may express a similar antibiotics pattern if they share in a common pool of R-factor plasmids (Spanggard et al., 1993). All isolates were sensitive to ciprofloxacin and showed resistance to rocephin and zinnacef except Micrococcus sp.

This study therefore confirms the presence of some pathogenic bacterial and fungal isolates from the frozen and smoked fish which are of public health significance and indicative of health risk in contacting diseases associated with these organisms. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be enforced.

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