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Safety Concerns on Microbes Associated with Fresh and Smoked Fish Sold in Igbokoda Fish Market, Nigeria

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Authors' contributions

This work was carried out in collaboration amomg the 3 authors.All authors read and approved the final manuscript.

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ABSTRACT

Fishes are important source of food for human globally because man gets a lot of minerals, vitamins, lipids and proteins from fishes and theirs products. Microbiological quality of fish is important to public health as indicative parameters to ensure safe consumption of fish by man. This study was carried out in order to evaluate microbes associated with fresh and smoked fishes on sales at Igbokoda fish market and their antimicrobial susceptibility profiles. Ten different fresh and smoked fish samples were collected from different fish-sellers at different selling points in Igbokoda fish market, aseptically using sterile containers. Samples from the skin, intestine and gill were obtained and cultured into three different media (MacConkey Agar, Nutrient Agar, *Salmonella-Shigella* Agar) for bacteria isolation and characterization. Other samples from the skin, intestine and

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gill were also cultured into Potato Dextrose Agar for fungal isolation and characterization. After 48 hours, on examination the total bacterial counts obtained from the skin, intestine and gill ranged between 2.4 to 8.7 x 10^6 cfu/ml while the total fungal counts ranged between 4.6 to 9.2 x 10^4 cfu/ml. Highest microbial load was obtained from the skin of the fish samples (7.2 x 10^6 cfu/ml), while the gill had the lowest microbial load (4.6 x 10^4 cfu/ml). The bacteria species isolated from the fish samples and their percentage of occurrence were; *Aeromonas hydrophilia* (11.2%)*, Bacillus species* (6.8%*)*, *Citrobacter freundii* (3.3%), *Escherichia coli* (23.2%), *Enterobacter cloacae* (6.1%), *Enterococcus faecalis* (6.5%), *Listeria monocytogenes* (4.5%), *Pseudomonas aeroginosa* (8.2%), *Salmonella species (*10.2%), *Staphylococcus aureus* (16.5%) and *Streptococcus species* (3.5%). The fungi isolated and their percentage of occurrence were*; Penicillium species* (16.2%), *Aspergillus flavus* (8.4%), *Aspergillus niger* (25.5%), *Candida species (*16.4%*)*, *Fusarium species* (6.5%), *Mucor species* (8.6%), *Rhodotorula species* (7.2%) and *Rhizopus stolonifer* (11.2%). Antibiotics susceptibility testing showed that all bacteria isolates were susceptible to Chloramphenicol, Ciprofloxacin, Augmentin, and Amoxillin. While *Salmonella species*, *Staphylococcus aureus* and *Streptococcus species* showed strong resistance to Septrin, Gentamycin and Streptomycin but intermediate susceptible to Erythromycin, Ampiclox, and Tetracycline. The antifungal susceptibility testing of the fungal isolated from fish samples showed that all the isolates were susceptible to all the antifungal agents used except *Candida* species that showed little resistance to clotrimazole. The study showed that microbial loads in the fish samples were low and, also there was no presence of multidrug resistant bacteria in sampled fish; therefore the fishes were safe for consumption. Although, it is recommended that good processing method should be adopted by fish handlers to prevent health risks to the fish consumers.

Keywords:Fungi; bacteria; antibiotics; antifungal; health risks; susceptible; resistance.

1. INTRODUCTION

Fishes are important source of food for human globally because man gets a lot of minerals, vitamins, lipids and proteins from fishes and theirs products. However, availability of these vital nutrients depends to a large extent on the methods of preservation such as salting, roasting, drying, and freezing [1,2,3]. Fresh and smoked fishes of different types are of great demands by the consumers in Nigeria because they are relatively cheaper source of animal protein. Fish and fish products are important, not only from the nutritional point of view but also as a source of income and revenue to the sellers and the government respectively.

Fishes are perishable, high-protein foods that typically contain a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, cadaverine, organic acids, ketones and sulfur compounds [4].

Fishes are regarded as omnivorous animal because they feed on plants and other small sea animals of water bodies. Some of the varieties of fishes which are available in the world are as follows: Siamese fighting fish, Gold fish, Guppy, Blow fish, Common carp, Snakehead murrel, Nile tilapia, Ocean sunfish, Oscar, Wels catfish, Sucker-mouth catfish, Northern pike, Freshwater angelfish, Asian arowana, Blue tang, Neon tetra, Swordfish, Common molly, Stonefish, Barramundi, Giant oarfish, Bluegill, Mahi mahi, Whale, shark, Rainbow trout, Atlantic salmon, Basa, Zebra fish, Frilled shark, Giant, Megalodon, Burbot, and Garfish [5].

Microbiological quality of fish and its products is of great importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. The freshwater or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animals and plants sources [6]. The fish tissues and organs, including the skin, olfactory system, gills and also the gut are in direct contact with the environment and thus are the first contact points of the microbes with the fish. Although, mucus covering fish tissues and organs can be considered as a primary defense line against pathogens and unfavourable environmental factors. The mucus contains immune components such as lectins, complement proteins, antimicrobial peptides, immunoglobulins, lysozymes and a variety of other enzymes, including proteases [7]. The mucus provides a carbon source for commensal microbes that can subsequently form a protective shield against invading pathogens [8,9]. The mucus of the fish skin and gills generally

contains more aerobic than anaerobic microbes [9,10].

It is very difficult to estimate and compare fish microbiomes; the fish skin typically harbors about 10^4 bacteria per cm^2, whereas the gills harbor about 10^6 bacteria per gram of tissue based on cultivation-based methods [9,11,12].

The composition of microbiome of the gills and skin is different; the protected niches of the gill lamellae contain more microbes that somehow favor gas exchange [9,13]. For example, the gill microbiota of rainbow trout (*Oncorhynchus mykiss*) contains mostly *Proteobacteria* and *Bacteroidetes, the* intestine contains *Flectobacillus* and *Flavobacterium*, while the skin contains more *Actinobacteria* and *Firmicutes"* [10,13]. A recent study conducted by Awe and Adejo [9] showed that the gills fresh obtained from river Niger, Lokoja, Nigeria contained different flora of bacteria and fungi. The bacteria are thought to play important role in defence mechanism of these fish, and also play an important role in detoxifying the excreted ammonia [9,14,15]. Derived from cultivation-based methods; the fish intestine generally harbours up to 10^8 aerobic heterotrophic bacteria represented by approximately 500 species and up to 10^5 anaerobic bacteria per gram of gut tissue [9,16]. For most fish species, the most abundant phyla found in fish guts are typically Proteobateria, Actinobacteria and Bacteroidetes [9,10,17]. Studies showed that skin, gills and intestine of smoked and fresh fishes contain members of the major bacterial phyla and the fungi. The microbial community of fish is much more diverse [8,9].

Fishes and other aquatic organisms are prone to environmental hazards caused by water pollution as a result of human activities. Although infection as a result of microbial contamination of fish and its products may not usually cause disease or diseases to the consumers however, environmental factors may upset the balance between the potential pathogens and their hosts [18,19].

The public health significance of fish contamination lies not only in their ability to cause diseases but also their possible role in the transfer of antibiotic resistant strains to other pathogens. Cross-contamination of household utensils and other foods by such fishes could aid the spread infection at home. Therefore maintenance of quality of fish is of utmost importance in production and trade of fish and fish products.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Ten different fresh and smoked fish samples were collected from different fish-seller at ten different points in Igbokoda fish market, aseptically using sterile containers. The collected samples were preserved with ice-pack box. Also ten different smoked samples were purchased from different retailers at different point in Igbokoda market as well and kept in sterile polythene bags. And both samples were transported to Adekunle Ajasin University Akungba-Akoko, Microbiology laboratory for micobiological analyses. The fish specie collected for analysis were; Catfish, *Tilapia zilli,* Stingray fish, Butterfly fish and African knifefish.

2.2 Isolation and Enumeration of Microorganisms

The fresh fish samples collected were removed from the container. The samples were subjected to laboratory examination within the first one hour on arrival to the laboratory. Using sterile knife, samples were aseptically dissected to remove the gills, intestine, while the skin was scraped, using the procedures described by Walke et al. [13]. The different parts collected were then blended for homogeneity and 5 g of each sample was taken for analysis. Also the smoked samples were blended and 5 g of each sample was taken for analysis as well, to obtain uniform distribution of cells through the culture, the blended fish samples were aseptically serially diluted. One milliliter of 10^{-6} dilution of each sample was inoculated on Nutrient agar (for total Viable bacteria), MacConkey agar (for coliform), *Salmonella-Shigella* agar (for *Salmonella* and *Shigella)* and, for fungi; 1 ml of 10^{-4} dilution of each sample was inoculated on potato dextrose agar containing 0.1% streptomycin using pour plate technique. The plates were prepared in triplicates and incubated under aerobic condition at 37°C for 24 - 48 hours, with the exception of potato dextrose agar plates which were incubated at 28°C, each plate was counted using the Quebec colony counter (Reichert, USA) and expressed as colony forming unit per ml of sample homogenate (cfu/ml) [20].

2.3 Methods for bacterial Identification

The isolates were sub-cultured and characterized based on colonial morphology, cellular morphology, Gram staining reactions and biochemical tests. Identification was done using Bergey's Manual of determinative bacteriology [21].

2.4 Morphological Characterization of the Fungi

Microscopic Identification: For microscopic Identificationa thin smear was prepared in accordance to [22] by "emulsifying a loopful of an isolate under test on a clean slide with a drop of water. The film was spreading to make a thin film and then air dried after which it was stained with a lactophenol cotton blue and then observed with a light microscope under X10 and X40 objective lenses.

Macroscopic Identification: The macroscopic Identification of the fungi was carried out after isolation on Yeast Malt Agar (YMA) and Potato Dextrose Agar (PDA). These features included morphology, surface characteristics, presence of pseudohyphae, hyphae, ascospore formation, and vegetative reproduction [23,24]. The microscopic and cultural features of organism identified were noted and compared with the yeast database (https://theyeasts.org).

2.5 Biochemical Characterization of Fungi Isolates

Oxidation-Fermentation (O-F) tests: Fungi Nitrogen Base (Difco) (FNB) broth (the base contains all essential nutrients and vitamins necessary for the cultivation of Fungi except a source of carbon) was prepared by adding 6.7 g of base and 5 g of carbohydrate to 100 ml of distilled water (warmed). The preparation was thoroughly mixed, filter sterilized and stored in refrigerator at 4°C for a maximum of one week before use. An inoculum culture of the test organisms was grown on PDA plates for two days at 25°C and used to inoculate the FNB carbohydrate broth medium which was incubated at 25°C for 5 days. After incubation, the glass tubes were shaken and released to determine whether growth had occurred. The carbohydrates tested using FNB were glucose, sucrose, maltose, xylose, galactose, lactose, raffinose, melibiose, mannitol, and trehalose [22,24].

2.6 Antibiotics Sensitivity Test

Bacteria: The antibiotics susceptibility of the isolates was determined by the disc diffusion method on Muellar Hinton Agar. The antibiotic multi-disc; made in Nigeria by Maxicare Medical Laboratory, containing both Gram negative (-ve) and Gram positive (+ve); Septrin, Chloramphenicol, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Streptomycin, Ampiclox, Amoxicillin, Streptomycin and Erythromycin, were used. The inoculum was standardized by adjusting its density to equal to a barium sulphate ($BaSO_4$) at 0.5 McFarland turbidity standards, and then incubated at 37°C for 18hrs. The diameter of the zone of inhibition was measured in millimeter (mm) [25].

2.7 Antifungal Susceptibility Testing

The antifungal susceptibility of fungi species isolated from fishes samples was determined by disc diffusion method on glucose methylene blue Muller Hinton agar. Antifungal Susceptibility Test of Yeasting method was used as explained by CLSI guidelines M44-A [26].

Antifungal agents used were Amphotericin-B (100µg), Fluconazole (25µg), Ketoconazole (50µg) and Clotrimazole (50µg). Glucose methylene blue Mueller Hinton agar (GM-MH) was prepared by addition of 2% glucose and 0.5µg of methylene blue to Mueller Hinton agar. The inoculum was prepared by picking four distinct colonies of approximately 1mm from 24 hours old cultures grown on Sabouraud's dextrose agar (SDA). Colonies were suspended in 5ml of sterile 0.85% saline. This suspension was vortexed to adjust the turbidity yielding 1×10^6 to 5×10^6 cells/ml and streaked on the entire surface of GM-MH agar the antifungal discs were placed 24mm apart from each other. The plates were then incubated at 37^{o}C for 24 hours the plates were read after 48hours.zone diameter were interpreted as per the approved CLSI M44-A guidelines.

2.8 Statistical Analysis

The data generated were analyzed by one way ANOVA. All data were expressed as Mean ± SEM. P values less than 0.05 was considered to statistically significant [27].

3. RESULTS

Table 1 shows the viable colony count isolated from different parts of fresh fish samples

collected from Igbokoda fish market. Out of the three different parts of the fish samples examined, highest number of viable colony counts were obtained from the fish skin (8.7 x 10^6 cfu/ml) while the least number of viable colony counts were obtained from the fish gills (2.6 x 10^6 cfu/ml). Thirty distinct isolates were obtained from the different samples from the primary plates. The viable colony counts (cfu/ml) of all the fish samples showed that smoked fish samples had higher colony count than that of the fresh fish samples as shown in Table 2. The total fungal count of fresh fish samples collected from Igbokoda fish market in Igbokoda is shown in Table 3. The highest total fungal count was obtained from the intestines of samples fish (6.2 × 10^4cfu/ml); while lowest total fungal count was obtained from gills of the samples fish (3.3 × 10^4 cfu/ml). In all the parts of fish sampled, the highest microbial loads were obtained from the skins (Fig. 1). The macroscopic and microscopic identification of fungi associated with fresh and smoked fish samples indicated a total of eight fungal isolates were identified. These include *Penicillium species, Aspergillus flavus, Aspergillus niger, Candida species, Fusarium species, Mucor species, Rhodotorula species* and *Rhizopus stolonifer*. The frequency of prevalence of fungi associated with fresh and smoked fish samples is shown in Fig. 2. The most prevalent fungus in smoked fish was *Aspergillus niger* (28) while the least prevalent was *Fusarium species* (4). While the most prevalent fungus in fresh fish was *Candida species* (19*)* and least prevalent was Mucor species (2).

The identified bacteria obtained from the fish samples include; *Aeromonas hydrophilia, Bacillus species, Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella species, Staphylococcus aureus* and *Streptococcus species. Escherichia coli* were the most prevalent bacteria isolated from the samples especially the smoked fish samples followed by *Staphylococcus aureus* (Fig. 3).

All the fungi isolates were susceptible to Amphotericin-B, Ketoconazole, Fluconazole, Clotrimazole, except *Candida* species that exhibited resistance to Clotrimazole as shown in Table 7.

The result of the antibiotic susceptibility tests for the bacterial isolates was presented in Table 8. Antibiotic susceptibility pattern showed that all bacteria isolates were susceptible to Chloramphenicol, Ciprofloxacin, Augmentin, and Amoxillin, *Salmonella* species*, Staphylococcus aureus* and *Streptococcus species* showed strong resistance to Septrin, while Gentamycin and Streptomycin but intermediately susceptible to Erythromycin, Ampiclox, and Tetracycline.

4. DISCUSSION

The results of the microbes associated with fresh and smoked fishes on sale at Igbokoda fish market in Ilaje local government area of Ondo State, Nigeria indicated that there are highest microbial counts in the skin samples compared to the gill and intestine. This may be attributable to the handling and processing techniques. The gills had the lowest microbial population compared to the intestine and skin in all the samples analyzed. According to Ezeri et al. [14] the numbers of bacteria associated with the gills are actively maintained at low level, thereby

Table 1. Total bacterial viable counts of the fresh fish samples collected Igbokoda fish market

Part of fish samples	Viable colony count (cfu/ml)
Skin	7.2 x 10^6
Gills	2.6 x 10^6
Intestine	6.6 x 10^6

Table 2. Total bacterial viable counts of the smoked fish samples collected from Igbokoda fish market

Part of fish samples	Viable colony count (cfu/ml)
Skin	8.7 x 10^6
Gills	4.0 x 10^6
Intestine	6.2 x 10^6

Table 3. Total fungal counts of fresh fish samples collected from Igbokoda fish market

Parts of fish samples	Viable colony count (cfu/ml)
Skin	5.3 x 10^4
Gills	3.2 x 10^4
Intestine	6.2 x 10^4

Table 4. Total fungal counts of smoked fish samples collected from Igbokoda fish market

Parts of fish samples	Viable colony count (cfu/ml)
Skin	9.2 x 10^4
Gills	3.3 x 10^4
Intestine	4.2 x 10^4

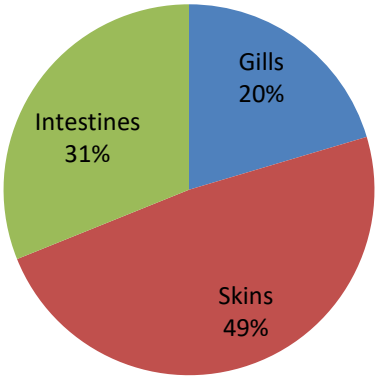

Fig. 1. Percentage of microbial loads in the various parts of the fish samples collected from Igbokoda fish market

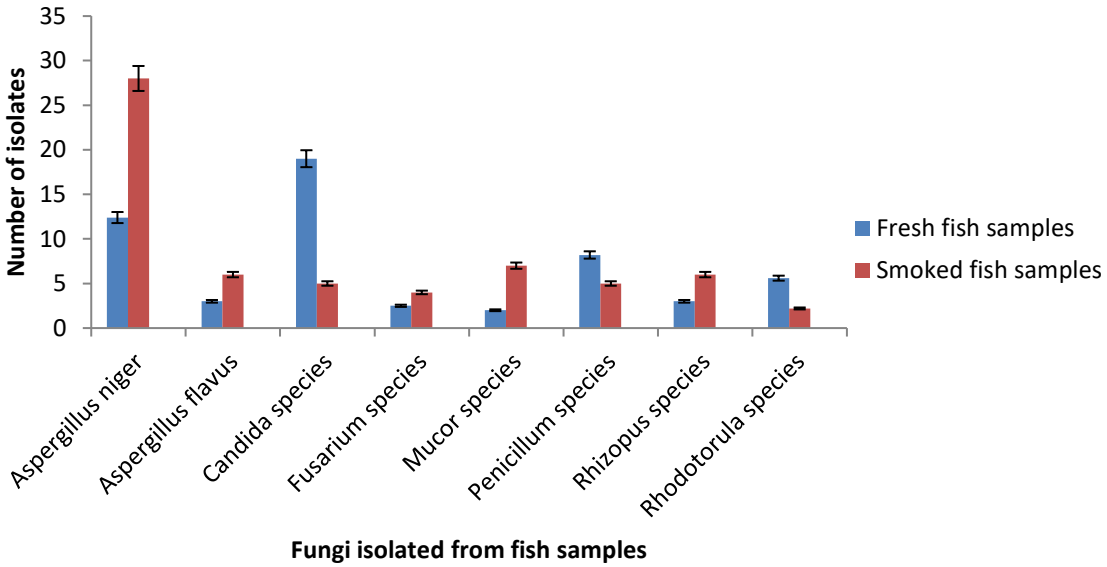

Fig. 2. Prevalence frequency of fungi isolated from fresh and smoked fish samples collected from Igbokoda fish market

implying that fish probably had mechanism which enables it to keep the number of microoganisms low, and therefore afford it some degree of protection against microbial infections. Research conducted by Walke et al. [13] revealed some opportunistic pathogens in the mucus of skin, gills, fins and mouth of Labeorohita. The presence of *Staphylococcus aureus* in all the samples analyzed (Fig. 3) can be attributed to human contact during handling and processing because *S. aureus* is normal body flora of human's skin [28,29]. However, *Staphylococcus aureus* produces a variety of extracellular enzymes and toxins that have been found to be responsible for food poisoning and can rapidly develop resistance to many antimicrobial agents and pose therapeutic problems [9,10].

Also, *Aeromonas hydrophilia, Bacillus species, Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella species,* and *Streptococcus species* have been implicated in fish-borne diseases of humans [9,30]. *Salmonella species* has been demonstrated to cause enteritis and systemic disease. The presence of these microorganisms in the

samples of fish analysed constitutes a food safety concern because fishes could be potential agent of transfer of these species to unsuspecting consumers. *Streptococcus species* that have been found to be associated with aquatic contamination include *Streptococcus pyogenes* and *Streptococcus pneumonia*. The presence of *Escherichia coli* in all the samples analyzed as shown in Fig. 3 may be due to its ubiquitous nature as it could be found in all environments including human skin, water and air during processing [4]. This result corroboratedthat of [17] who observed that most of the bacteria flora associated with spoilage of fish were Gram negative rod bacilli

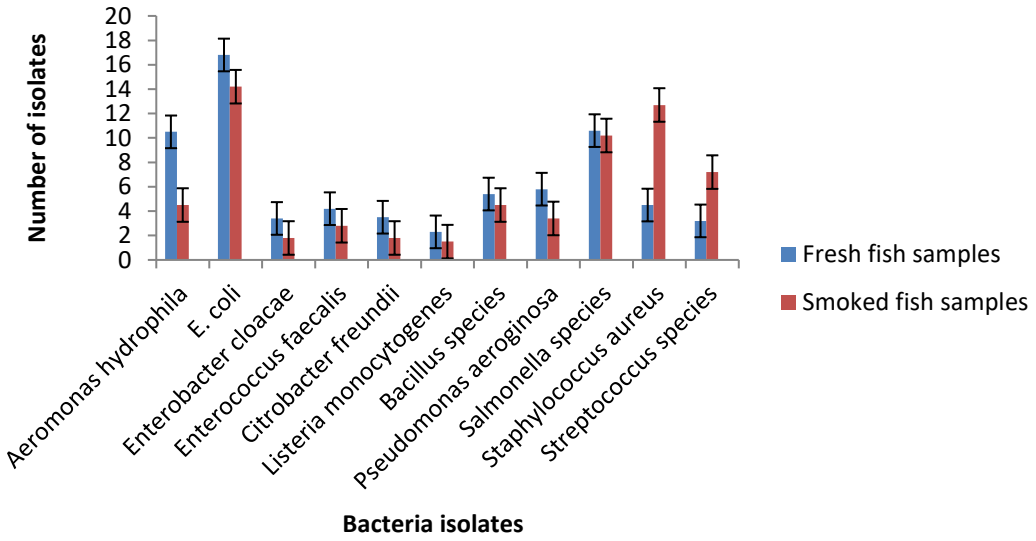

Fig. 3. Prevalence frequency of isolated bacteria from fresh and smoked fish samples collected from Igbokoda fish market

Table 5. Cultural and morphological characterization of the fungi isolated from fresh and smoked fish samples collected from Igbokoda fish market

Description	Probable Fungi isolates
The colony was white and woolly. The hypha were thick and non-septate, columella were round. The sporagiopores departs laterally from mycelium.	*Muccor*spp
Colonies are whitish-cream in color, smooth, glabrous and yeast-like in appearance. Presence of spherical to sub spherical blastoconidia.	*Candida albicans*
The colony contains black conidiophore. Conidial heads, radiate. Conidiophorestipe smooth-walled, hyaline with brown colour	*Aspergillusniger*
Colonies have yellow-green conidiophores. Conidiophores have stipes with smooth-walled hyaline with brown colour.	*Aspergillusflavus*
Colonies have aerial mycelium with whitish or peach colour; Conidiophores are usually short branched on phialides.	*Fusarium*spp
Colonies have whitish color becoming grayish-brownish. Sporangiophores are dark brown, rough-walled stolons opposite the branched rhizoids. It has sporangia with sub-globose, ovoid, with blackish-brown color after 48 hours	*Rhizopusstolonifera*
Colonies grow and sporulate with yellow or brown-green conidiophores with 3-6 phalides. Phalides often solitary, cylindrical with a short neck	*Penicillum*spp
Soft, moist and oval-shaped cells that gives pink colonies on yeast malt agar, growth rapidly within 24 hours of incubation.	*Rhodotorula*spp

Table 6. Phenotypic and biochemical characterization of bacteria isolated from fresh and smoked fish samples collected from Igbokoda fish market

Probable Bacteria isolates	Phenotypic and biochemical characterization of bacteria isolates											
	Colony edge	MT	Gram's staining	CT	CAT	OT	Gl	Sc	La	Ma	Fu	VP
S. aureus	E	+	+ve	+	+	-	+	+	+	+	+	+
E. coli	E	+	-ve	-	+	-	+	+	+	+	+	-
A. hydrophila	E	+	-ve	+	+	+	+	+	+	+	+	-
Bacillus species	L	+	+ve	+	+	+	+	+	+	+	+	+
Enterobacter cloacae	E	+	-ve	-	+	-	+	+	+	+	+	+
E. faecalis	E	-	+ve	-	-	-	+	+	+	+	+	+
Pseudomonas aeroginosa	L	+	-ve	+	+	+	+	-	-	-	-	-
Salmonella species	E	+	-ve	+	+	-	+	-	-	+	-	-
Listeriamonocytogenes	L	+	+ve	-	+	-	+	+	+	-	+	+
Citrobacterfreundii	E	+	-ve	+	+	-	+	-	+	+	+	+
Streptococcus species	E	-	+ve	+	-	-	+	+	+	-	+	-

Keys: L= lobate, E= entire, MT = motility test, CT = citrate test, CAT = catalase test, OT= oxidase test, VP = VogesProskauer, Gl= Glucose, Su= Sucrose, Fu = fructose, Ma = Mannitol, La = Lactose, -ve = Gram negative bacteria, +ve = Gram positive bacteria, - =indicates no reaction, + = indicates there is reaction

Table 7. Antifungal susceptibility profile of fingi isolated from fish samples collected from Igbokoda fish market

Fungi isolates	Antifungal agents used			
	Amphotericin-B	Fluconazole	Ketoconazole	Clotrimazole
Aspergillusniger	S	S	S	S
Aspergillusflavus	S	S	S	S
Candida species	S	S	S	R
Fusarium species	S	S	S	S
Mucor species	S	S	S	S
Penicillum species	S	S	S	S
Rhizopusstolonifera	S	S	S	S
Rhodotorula species	S	S	I	S

Keys: S = susceptible, I = intermediate, R = resistance

Table 8. Antibiotics susceptibility profile of bacteria isolated from fish samples collected from Igbokoda fish market

Bacteria isolates	Antibiotics used									
	Chlo	Cipr	Amox	Aug	Gent	Stre	Ery	Sep	Amp	Tetr
S. aureus	S	S	R	S	I	S	S	S	R	S
E. coli	S	S	I	S	R	I	I	R	R	S
Aeromonashydrophila	S	S	S	S	S	S	S	S	S	S
Bacillus species	S	S	S	S	S	S	S	S	S	S
Enterobacter cloacae	S	S	S	S	R	S	I	I	R	R
Enterococcusfaecalis	S	S	S	S	S	S	S	S	S	S
Pseudomonas aeroginosa	S	S	S	S	S	S	S	S	S	S
Salmonella species	S	S	I	S	R	R	R	R	R	R
Listeriamonocytogenes	S	S	S	S	S	S	S	I	I	R
Citrobacterfreundii	S	S	S	S	S	S	S	S	S	S
Streptococcus species	S	S	I	S	R	R	R	R	R	S

Key: Chlo = chloramphenicol, Cipr = ciprofloxacin, Amox = amoxillin, Aug= augmentin, Gent = gentamycin, Stre = streptomycin, Ery = erythromycin, Sep = septrin, Amp – ampiclox, Tetr – tetracycline, S = susceptible, I = intermediate, R = resistance

such as *E. coli, Salmonella species* and *Pseudomonas species.* The presence of *Enterococcus faecalis* and *Salmonella species* in all the fresh fish samples is an indication that the river where the fishes were sourced from was faecally contaminated. The presence of Bacillus species was not surprising since fish lives in water habitat full of microorganism confirmed that bacteria flora associated with a Nigeria rivers analysed include the genera *Bacillus, Lactobacillus, Staphylococcus, Escherichia, Micrococcus, Proteus* and others [9,10]. Bacillus species are implicated in causing a wide range of infectious diseases such as abscess, septicemia, wound and food borne infections, ear infections, meningitis, ophthalmitis, osteomyelitis, peritonitis and respiratory and urinary tract infections [31].

The fungal species isolated in the fresh and smoked fishes include; *Penicillium species, Aspergillus flavus, Aspergillus niger, Candida species, Fusarium species, Mucor species, Rhodotorula species* and *Rhizopus stolonifer*. All these fungi may come from the water habitat, environment, materials used in fishing and the handlers. The presence of *Mucor species Penicillium species, Aspergillus niger, Aspergillus flavus, Fusarium species* and *Rhizopus stolonifer* in the fish samples is not surprising as they disperse in the form of spores which is abundant in the environment and can be introduced through dust and soil [32]. Their presence in these fish samples is of serious public health concern as these fungi have all been implicated with the production of mycotoxin [33]. Ike et al. [7] Tudor et al. [34] reported that certain species of *Aspergillus* produced toxic metabolites, while *Mucor species* could degrade the biochemical structure of proteins and lipids thereby affecting the organoleptic property of the fish [5]. Antibiotic susceptibility pattern showed that all bacteria isolates were susceptible to Chloramphenicol, Ciprofloxacin, Augmentin, and Amoxillin. While *Salmonella species, Staphylococcus aureus* and *Streptococcus species* showed strong resistance to Septrin, Gentamycin and Streptomycin but intermediate susceptible to Erythromycin, Ampiclox, and Tetracycline. This result corroborated that of [9] that observed antibiotics resistance in *Salmonella species, Staphylococcus species* and *Streptococcus species* isolated from fresh fish samples. The result of the antimicrobial susceptibility profiles in this study is of public health concern, considering the fact that some of bacteria isolates were resistance to commonly used antibiotics and infection caused by these organisms may be difficult to treat, which can spread in populations resulted to disease out-breaks.

5. CONCLUSION

The results of the microbes associated with fresh and smoked fishes on sale at Igbokoda fish market in Ilaje Local Government Area of Ondo State, Nigeria indicated that there is diverse microbiota in the fish samples. The presence of these microorganisms in these samples constitutes a food safety concern because fishes could be potential agent of transfer of these microorganisms to unsuspecting consumers. The result of the antimicrobial susceptibility profiles in this study has public health implication considering the fact that some of bacterial isolates showed resistance to commonly used antibiotics. Health services could exercise caution on the indiscriminate and inappropriate use of antibiotics, and related compounds on animals and humans. Although ciprofloxacin and augmentin were effective against the isolates in this study, periodic monitoring using antibiograms is necessary to detect any changes in resistance patterns over time.

6. RECOMMENDATION

It is recommended that good processing method should be adopted by fish handlers to prevent health risks to the fish consumers. Also antimicrobial mapping method for individual can be adopted to control antibiotics and antifungal resistance in the population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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