Original Research Article

Characterization and Antimicrobial Susceptibility Profiles of Microbiomes Associated with Fresh and Smoked Fish on Sales at Igbokoda fish market, Ondo State, Nigeria.

ABSTRACT

Fishes are important source of food for human globally; man gets a lot of minerals, vitamins, lipids and proteins from fishes and theirs products. Microbiological quality of fish is importance to public health as indicative parameters to prevent fish spoilage and ill-health in man. This study was carried out in order to evaluate microbiome of 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 point in Igbokoda fish market, aseptically using sterile containers. Samples from the skin, intestine and gill were obtained and cultured in four different media; MacConkey agar, nutrient agar, *Salmonella-Shigella* agar and potato dextrose agar, and on examination the total bacterial count obtained from the skin, intestine and gill ranged between 2.4 to 8.7 x 10^6 cfu/ml while the total fungal count 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 has the lowest microbial load (4.6 x 10^4 cfu/ml). The bacteria species isolated from the fish samples were; *Aeromonas hydrophilia, Bacillus species, Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeroginosa, Salmonella species, Staphylococcus aureus* and *Streptococcus species.* The fungi isolated include*; Penicillium species, Aspergillus flavus, Aspergillus niger, Candida species, Fusarium species, Mucor species, Rhodotorula species* and *Rhizopus stolonifer*. 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 the presence of multidrug resistant bacteria in these fish samples and may pose a serious health concern to the consumers. It is therefore recommended that adequate measure should put in place to control water pollution cause by sewage and wastes, also good processing method should be adopted by fish handlers to eliminate health risks to the fish consumers.

Key words: Fungi, bacteria, antibiotics, antifungal, health risks, resource, microflora.

INTRODUCTION

Fishes are important source of food for human globally; 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 storage such as salting, roasting, drying, and freezing (Gram and Huss, 2001; Whong *et al*., 2003; Awe and Adejo, 2018). Fresh and smoked fishes of different types are of great demands by the consumers world-wide because it is relatively cheaper source of animal protein. Fish and fish products are importance, not only from the nutritional point of view but also as a source of income and revenue (Mahendra *et al*., 2016).

Fish is a very perishable, high-protein food that typically contains 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 (Dalgaard *et al.*, 2006).

Fishes are an omnivorous group 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, Goldfish, Guppy, Blobfish, Common carp, Snakehead murrel, Nile tilapia, Ocean sunfish, Oscar, Wels catfish, Suckermouth 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, Zebrafish, Frilled shark, Giant snakehead, Megalodon, Burbot, and Garfish (www.purdue.edu, 2023)

Microbiological quality of fish and its products is of 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 (Shinkafi and Ukwaja, 2010). 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 defence line against pathogens and unfavourable environmental factors. The mucus contains immune components like lectins, complement proteins, antimicrobial peptides,

immunoglobulins, lysozymes and a variety of other enzymes, including proteases (Brinchmann 2016; Ike et al., 2020). The mucus provides a carbon source for commensal microbes that can subsequently form a protective shield against invading pathogens (Ibemenuga and Okeke, 2014; Ike *et al*., 2020). The mucus of the fish skin and gills generally contains more aerobic than anaerobic microbes (Awe and Adejo, 2018; Jesumirhewe and Ibeh, 2020).

It is 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 (Kumar et al., 2007; Novotny et al., 2004; Awe and Adejo, 2018). 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 (Walke *et al*., 2015; Awe and Adejo, 2018). For example, the gill microbiota of rainbow trout (*Oncorhynchus mykiss*) contains mostly Proteobacteria and Bacteroidetes (Flectobacillus and Flavobacterium), while the skin contains more Actinobacteria and Firmicutes (Walke *et a*l., 2015; Jesumirhewe and Ibeh, 2020). A recent study conducted by Awe and Adejo (2018) 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 (Ezeri, 2001; Bundiati *et al*., 2011; Awe and Adejo, 2018). 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 (Wogu and Maduakor, 2010; Awe and Adejo, 2018). For most fish species, the most abundant phyla found in fish guts are typically Proteobacteria, Actinobacteria, and Bacteroidetes (Abu and Wondikom, 2018; Awe and Adejo, 2018; Jesumirhewe and Ibeh, 2020). 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 (Ike *et al*., 2020; Awe and Adejo, 2018).

Fishes and other aquatic organisms are prone to environmental hazards. Although infection as a result of microbial contamination may not usually result in disease, however, environmental stresses may upset the balance between the potential pathogens and their hosts. Some of these microbes may not be pathogenic for the fish but when they are consumed by man, a disease condition may occur (Abu and Egenonu. 2008; Ademola *et al.,* 2009).

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 pathogen. 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.

Materials and Methods

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. 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 processed.

Processing of Samples

The fresh fish samples collected were removed from the containers and each divided into three portions. One portion was subjected to laboratory examination within the first one hour on arrival to the microbiological laboratory; the second portion was examined after four hours under room temperature, and the third portion was examined above eight hours after allowing its ice to melt under room temperature in 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*., (2015). The different parts collected were then blended for homogeneity and 5 g of each sample was taken for analysis. Also the roasted 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.

Isolation and Enumeration of Microorganisms: 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 fungal 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) (Clarence *et al*., 2009).

Methods for bacterial Identification

The isolates were sub-cultured and characterization was based on colonial morphology, cellular morphology, Gram stainng reactions and biochemical reactions test. Identification was done using Bergey's Manual of determinative bacteriology (Cheesebrough, 2006).

Morphological characterization of the Fungi

Microscopic Identification: For microscopic Identificationthin a thin smear was prepared in accordance to Oyeleke and Manga (2008) 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 were determined 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 (Adetuwo, 2020; Sawalha, 2014). The microscopic and cultural features of organism identified were noted and compared with the yeast database (https://theyeasts.org).

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 (Oyeleke and Manga, 2008; Sawalha, 2014).

Antibiotics sensitivity Test

Bacteria: The antibiotics susceptibility of the isolate will be 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 (BaS04) 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) (Cheesbrough, 2006; Andrews, 2007).

Antifungal susceptibility testing

The antifungal susceptibility of *Candida* species isolated from clinical 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 (2010).

Antifungal agents used

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 48 hours.zone diameter were interpreted as per the approved CLSI M44-A guidelines.

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 (Ogbiebu, 2015)

Results and Analysis

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 (8.7×10^6 cfu/ml) while the least number of viable colony counts were obtained from the fish gills (2.6×10^6 cfu/ml). Thirty distinct isolates were obtained from the different isolates observed from the primary plates. The viable colony count (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 (6.2×10^4 cfu/ml); while lowest total fungal count was obtained from gills of the samples fish (3.3×10^4 cfu/ml). 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 isolated fungi associated with fresh and smoked fish samples is shown in figures 1. The most prevalent fungus in smoked fish was Aspergillus niger (28) and the least prevalent was *Fusarium species* (04). While the most prevalent fungus in fresh fish was *Candida species* and least prevalent was Mucor species (02).

The identified bacteria obtained from the fish samples include; *Aeromonas hydrophilia, Bacillus species, Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeroginosa, Salmonella species Staphylococcus aureus* and *Streptococcus species. Escherichia coli* was the most prevalent bacteria isolated from the samples especially the smoked fish samples followed by *Staphylococcus aureus* as shown in table 2.

Table 1: Total bacterial viable colony count for the fresh fish samples collected Igbokoda fish market

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

Table 2: Total bacterial viable colony count for the smoked fish samples collected from Igbokoda fish market

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

Table 3: Total fungal count of fresh fish samples collected from Igbokoda fish market

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

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

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

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

Description	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. Conidiophore stipe smooth-walled, hyaline with brown colour	*Aspergillus niger*
Colonies have yellow-green conidiophores. Conidiophores have stipes with smooth-walled hyaline with brown colour.	*Aspergillus flavus*
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	*Rhizopus stolonifera*
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.

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	+	+	-	+	-	-	+	-	-
Listeria	L	+	+ve	-	+	-	+	+	+	-	+	+

monocytogenes												
Citrobacter freundii	E	+	-ve	+	+	-	+	-	+	+	+	+
Streptococcus species	E	-	+ve	+	-	-	+	+	+	-	+	-

Keys: L= lobate, E= entire, MT = motility test, CT = citrate test, CAT = catalase test, OT= oxidase test, VP = Voges Proskauer, 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

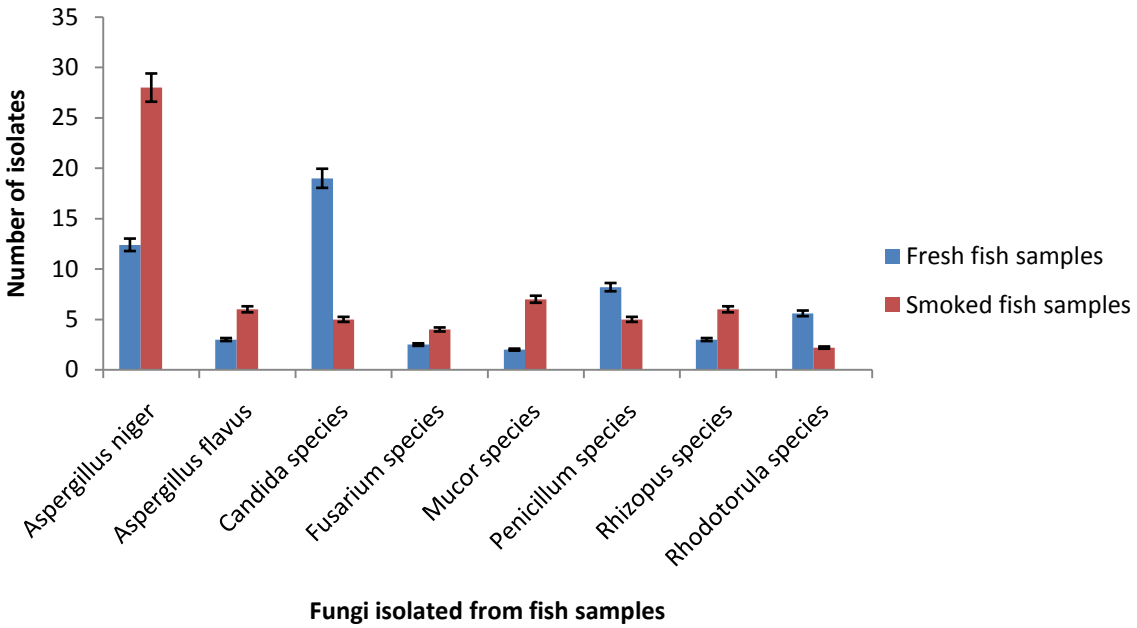

Figure 1: Prevalence frequency of isolated fungi from fresh and smoked fish samples collected from Igbokoda fish market

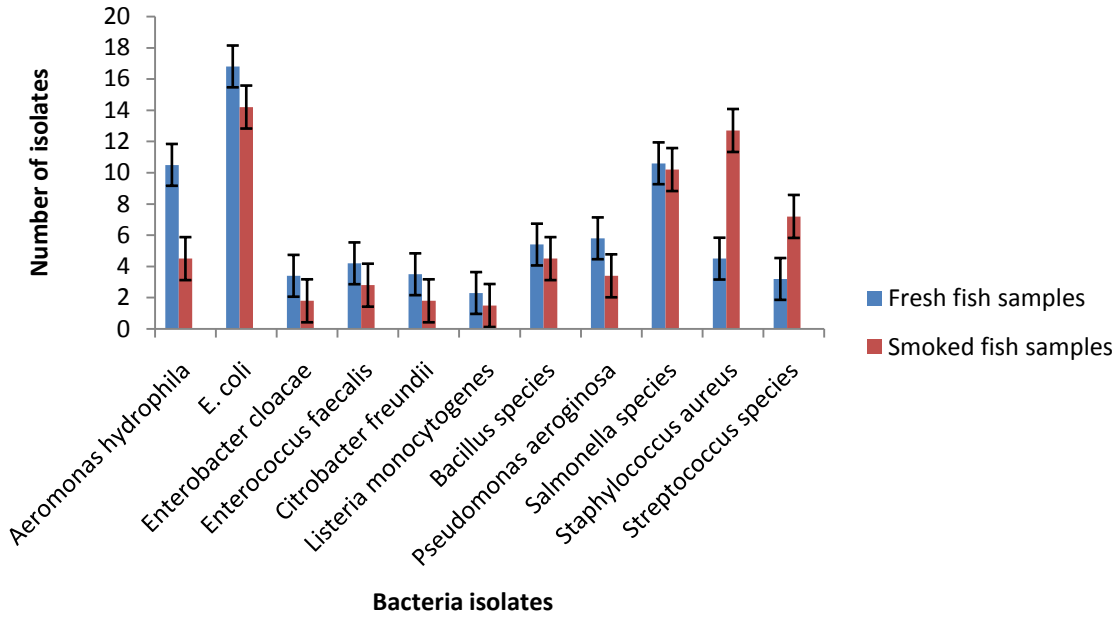

Figure 2: Prevalence frequency of isolated bacteria from fresh and smoked fish samples collected from Igbokoda fish market

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. While *Salmonella species, Staphylococcus aureus and Streptococcus species* showed strong resistance to Septrin, Gentamycin and Streptomycin but intermediate susceptible to Erythromycin, Ampiclox, and Tetracycline.

Table 7: Antifungal susceptibility profile of fingi isolated from fish samples collected from river Niger and markets in Lokoja

Fungi isolates	**Antifungal agents used**			
	Amphotericin-B	Fluconazole	Ketoconazole	Clotrimazole
Aspergillus niger	S	S	S	S
Aspergillus flavus	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
Rhizopus stolonifera	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 river Niger and markets in Lokoja

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
Aeromonas hydrophila	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
Enterococcus faecalis	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
Listeria monocytogenes	S	S	S	S	S	S	S	I	I	R
Citrobacter freundii	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

Discussion

The results of the microbiomes 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 highest microbial counts in the skin samples comparative to the gill and intestine. This may be attributable to handling and processing. The gills had the lowest bacterial population compared to the intestine and skin in all the samples analyzed. According to Ezeri et al. (2001), the number of bacteria associated with the gills are actively maintained at low level, thereby 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*., (2015) 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 can be attributed to human contact during handling and processing because S. aureus is normal body flora of human's skin (Granato, 2007). 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 (Awe and Adejo, 2018; Jesumirhewe and Ibeh, 2020).

Also, *Aeromonas hydrophilia, Bacillus species, Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeroginosa, Salmonella species,* and *Streptococcus species* have been implicated in fish-borne diseases of humans (Babu, 2000; Awe and Adejo 2018). 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 may be due to its ubiquitous nature as it could be found in all environments including human skin, water and air during processing. This result corroborated that of Abu, G.O and Wondikom (2018) who observed that most of the bacteria flora associated with spoilage of fish were Gram negative rod bacilli 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 (Awe amd Adejo, 2018; Jesumirhewe and Ibeh, 2020). Bacillus species are implicated in causing a wide range of infectious diseases including abcesses, bacterimia/septicaemia, wound and food borne infections, ear infections, endocardiatis, meningitis, ophthalmitis, osteomyelitis, peritonitis and respiratory and urinary tract infections (Morales *et al*., 2004).

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 (Apinis, 2003). Their presence in these fish samples is of serious public health concern as these fungi have all been implicated with the production of mycotoxin (Makun *et al*., 2009; Ike *et al*., 2020). Tudor et al. (2009) 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. 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 Awe and Adejo (2018) that observed multi-drug 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 multi-drug resistance was extremely common. Some of the resistant isolates are opportunistic pathogens and infection caused by these organisms may be difficult to treat, which can spread in populations resulted to disease out breaks.

Conclusion

The results of the microbiomes 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 present in the sampling fishes. 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. The result of the antimicrobial susceptibility profiles in this study has public health implications, considering the fact that multi-drug resistance was extremely common. 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.

Recommendation

As concerns about environmental contamination by humans, industrial and agricultural waste is on the increase, it is of importance to develop reliable mechanism that can be used to mitigate environmental pollution cause by indiscriminate dumping of wastes and domestic sewages. Also antimicrobial mapping method for individual can be adopted to control antibiotics and antifungal resistance in the population.

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