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Isolation and identification of pathogenic bacteria from fresh fish organs

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ABSTRACT

Aquaculture products can harbor pathogenic bacteria which are part of the natural microflora. Microbial pathogens associated with fish can be transmitted to human that uses the fishes as source of food or handling. This study aimed to isolate human pathogenic bacteria present in the gills, gut and skin of apparently healthy fishes. Tilapia (*Clarias lazera*) and *Oreochromicniloticus* (Cat fish) were collected from Gombe main market and Dadin-kowa of Gombe state. Bacteria were isolated from the fish skin, gill, and gut. Identification of the bacteria was conducted using biochemical tests on specific culture media. Different pathogenic bacteria were isolated from the fish species which appeared yellow or white in colour, small with pin head, while some are circular and irregular that spread all over the media.. The isolates were higher from Dadin-kowa samples compared to Gombe main market. The total bacteria count value ranges between 1.01 x 10^3 to 1.50 x 10^3 for the tilapia fish and 1.04 x 10^3 to 2.20 x 10^3 cfu/ml for cat fish. The identification results revealed that *Staphylococcus aureus* and *Eshcherichia coli* was the most abundant pathogenic bacteria. Presence of *Staphylococcus aureus* and *Eshcherichia coli* is attributed to contamination of the fish samples by man through handling and processing. These enteric bacteria from the fishes indicated the organisms faecal contamination and/or water pollution, as well, represents a potential hazard to humans' life.

Keywords Pathogenic bacteria, Tilapia, Cat fish, *Staphylococcus aureus*, *Eshcherichia coli*

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1. Introduction

Fish is the most important source of protein providing about 16% of animal protein consumed by the world populace. It is estimated that about one billion people worldwide rely on fish as their primary source of animal protein [1]. Fish are widely preferred food all over the globe and cheap source of protein in many developing nations[2]. At present there are over 20,000 known species of edible fish, shellfish and sea mammals, but sea food has long been association with transmission of diseases[3]. Microbiological quality of fish and shellfish has been a matter of great public health concern at both for domestic and international markets. The contamination harms includes the presence of pathogenic microorganisms which causes a lots of health problem to the consumers [4]. Equally, it involves other lower animals such as members of genus *Vibrio* that are native to aquatic environment and poses serious health hazard in humans as a result of consumption of raw or inadequately cooked seafood.

Sea foods usually harbors infective agents like pathogenic bacteria, which are present in the aquatic environment either naturally or coming through human operations [5]. In live and fresh fish, pathogenic bacteria may be associated with the gill portion, skin and gut [6]. Despite upsurge of knowledge on fisheries and its associated diseases, food borne diseases are perhaps among the most widely spread health problem which caused lots of economic meltdown. The available evidence clearly indicates that microbial contaminant is one of the major causes of food borne illnesses[7]. The high microbial load encountered in fish organ either skin, gut or gill, and the problem of food hazard posed by microorganisms together with the extreme susceptibility of aquaculture to pollution from domestic, industrial and agricultural discharge are the problem that prompt to this research. Therefore, the present study evaluated the presence of pathogenic bacteria in fresh Tilapia and cat fish sold at some outlets of Gombe city, Nigeria; focusing on their gut, gill and skin for isolation and identification of the pathogens.

2. Materials and Methods

2.1. Fresh fish sample collection

A total of 40 fresh fishes belonging to two species were purchased from two different locations (Gombe main market and Dadin-kowa) in Gombe state, Nigeria [8]. The fish species include Tilapia (*Oreochromisniloticus*) and Catfish (*Clariaslazera*) which purchased between 8am and 10am and immediately placed in thermal flask to transport to the laboratory for analysis. They were identified by their clarity and firmness eyes, color of gills and the seaweed smell as qualities for identifying fresh fish [9].

2.2. Isolation of pathogenic bacteria

Sterile media for isolation was prepared according to the manufacturer's instruction, while the other necessary components were made according to procedure as described [10]. 15g of peptone was dissolved in 1000ml and autoclaved at temperature of 121°C for 15 minute. The solution was allowed to cool and poured into test tubes, each containing 1 Omi of peptone water.

Fish organ including skin, gill, and gut was placed in a sterile tray disinfected with 70% ethanol and screened for bacterial contamination. The organs were swabbed separately with sterile cotton swab stick and inoculated into l0 ml peptone, then incubated overnight to observe the bacterial growth. Different media type was used for the isolation of pathogenic bacteria. For nutrient agar, 20g and 47g of MacConkey agar was dissolved in 1 litre of distilled water. The mixture were heat to dissolve the agar and autoclaved at 121°C for 15 minutes. Whereas, 58g of brilliant green was dissolved in 1000ml of distilled water and 25.8g of SMAC agar in 500ml of distilled water, then, at 121°C for 15mm. The overnight culture was sub-cultured in the agar for isolation.

2.3. Identification of pathogenic bacteria

Identification of the isolates was carried out by colony count, gram staining, and biochemical test according to Bergey's Manual of Systematic Bacteriology [11]. The use of biochemical tests, such as catalase, oxidase, coagulase, indole, citrate and mannitol test, which interpret the bacterial genus and species according to Clinical and Laboratory Standards Institute (CLSI) guideline, (CLSI, 2016) was carried out.

3. Results and discussion

3.1. Isolation and enumeration of bacteria

Forty samples of the fish from two different locations were investigated for the occurrence of pathogenic bacteria. Table 1 showed the total plate count result of the isolated bacteria obtained from the fresh fish samples. The colony count result showed that all the fish samples contain several types of colonies which appeared yellow or white in color. The forms of colonies are small with pin head, while some are circular and irregular which spread all over the media. The pathogenic bacteria load were much from Dadin-kowa fish compared to Gombe main market which comprises *Staphylococcus aureus*, and *Eshcherichia coli* (Table 4.1) likes. The isolation results revealed a total bacteria count value ranges between 1.01×10^3 to 1.50×10^3 for the tilapia fish and 1.04×10^3 to 2.20×10^3 cfu/ml for catfish. In this research, the gut of the catfish from Dadin-kowa has the highest number of bacteria. A high population of bacteria in food indicates the general

quality of the food and the degree of spoilage it might have undergone. The occurrence of total bacterial counts of the samples investigated having > $5X10^6$ CFU/ml raises concern about the hygienic status of production and point of sale environment. Although only a few infectious agents in fish are able to infect humans, some exceptions such as *Salmonella* exist that may result in fatalities [12, 13].

Table 1 Isolation and enumeration of bacterial species in skin, gill and gut of fishes from sampling stations.

Sample area	Fish species	No. of fish	Region	Pathogens identified from fish samples	Dilution factor	No. of colony	Population in cfu/ml
Gombe Main Market	Tilapia Oreochromis Niloticus	10	Skin	*Eshcherichia coli*	10^3	101	$1.01x10^3$
			Gills	*Staphylococcus aureus*	10^3	150	$1.50x10^3$
	Catfish Clariasl	10	Skin	*Eshcherichia coli*	10^3	104	$1.04x10^3$
			Gill	*Staphylococcus aureus*	10^3	100	$1.00x10^3$
Dadinkowa	Tilapia Oreochron	10	Skin	*Eshcherichia coli*	10^3	110	$1.10x10^3$
			Gill	*Staphylococcus aureus*	10^3	102	$1.02x10^3$
	Cat fish Clariasl	10	Skin	*Eshcherichia coli*	10^3	160	$1.60x10^3$
			Gill	*Staphylococcus aureus*	10^3	180	$1.80x10^3$
			Gut	*Eshcherichia coli*	10^3	220	$2.20x10^3$

3.2. Biochemical identification of the pathogenic bacteria

The isolated bacteria were identified to be gram positive and gram negative (rod and cocci). The gram positive cocci resemble *Staphylococcus* and the rod shape gram negative bacteria resemble *Eshcherichia coli* (Table 2 and 3). The results revealed that *Staphylococcus aureus* and *Eshcherichia coli* was the most abundant pathogenic bacteria associated with the fishes in Gombe. The presence of *Staphylococcus aureus* was attributed to the contamination of the fish samples by man through handling and processing as suggested by[14].

Table 2 Biochemical identification of tilapia fish (*OreohromicNilocicus*) from sampling stations.

Culture number	Region	Gram stain	Shape	Oxi	Cat	Coa	S.S	Ind	Cit	Main	Suspected organism
S_1	B_1T_1S	+	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	B_1T_1G	+		+	+	-	+	-	+	+	
	B_1T_1A	+		+	+	-	+	-	+	+	
S_2	B_1T_2S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_2G	+		+	+	-	+	-	+	+	
	B_1T_2A	+		+	+	-	+	-	+	+	
S_3	B_1T_3S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_3G	+		+	+	-	+	-	+	+	
	B_1T_3A	+		+	+	-	+	-	+	+	
S_4	B_1T_4S	+	Rod	+	+	-	+	-	+	+	*Escherichia coli*
	B_1T_4G	+		+	+	-	+	-	+	+	
	B_1T_4A	+		+	+	-	+	-	+	+	
S_5	B_1T_5S	+	Rod	+	+	-	+	-	+	+	*Escherichia coli*
	B_1T_5G	+		+	+	-	+	-	+	+	
	B_1T_5A	+		+	+	-	+	-	+	+	
S_6	B_1T_6S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_6G	+		+	+	-	+	-	+	+	
	B_1T_6A	+		+	+	-	+	-	+	+	
S_7	B_1T_7S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_7G	+		+	+	-	+	-	+	+	
	B_1T_7A	+		+	+	-	+	-	+	+	
S_8	B_1T_8S	+	Rod	+	+	-	+	-	+	+	*Escherichia coli*
	B_1T_8G	+		+	+	-	+	-	+	+	
	B_1T_8A	+		+	+	-	+	-	+	+	
S_9	B_1T_9S	+	Rod	+	+	-	+	-	+	+	*Escherichia coli*
	B_1T_9G	+		+	+	-	+	-	+	+	
	B_1T_9A	+		+	+	-	+	-	+	+	
S_{10}	$B_1T_{10}S$	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	$B_1T_{10}G$	+		+	+	-	+	-	+	+	
	$B_1T_{10}A$	+		+	+	-	+	-	+	+	

Table 3 Biochemical identification of catfish (*Clarias lazera)* sample from Gombe Main Market.

Culture number	Region	Gram Stain	Shape	Oxi	Cat	Coa	S.S	Ind	Cit	Main	Suspected organism
S_1	B_1T_1S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_1G			+	+	-	+	-	+	+	
	B_1T_1A			+	+	-	+	-	+	+	
S_2	B_1T_2S	+	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_2G			+	+	-	+	-	+	+	
	B_1T_2A			+	+	-	+	-	+	+	
S_3	B_1T_3S	-	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	B_1T_3G			+	+	-	+	-	+	+	
	B_1T_3A			+	+	-	+	-	+	+	
S_4	B_1T_4S	-	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	B_1T_4G			+	+	-	+	-	+	+	
	B_1T_4A			+	+	-	+	-	+	+	
S_5	B_1T_5S	-	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	B_1T_5G			+	+	-	+	-	+	+	
	B_1T_5A			+	+	-	+	-	+	+	
S_6	B_1T_6S	+	Rod	+	+	-	+	-	+	+	*Escherichia coli*
	B_1T_6G			+	+	-	+	-	+	+	
	B_1T_6A			+	+	-	+	-	+	+	
S_7	B_1T_7S	-	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_7G			+	+	-	+	-	+	+	
	B_1T_7A			+	+	-	+	-	+	+	
S_8	B_1T_8S	-	Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
	B_1T_8G			+	+	-	+	-	+	+	
	B_1T_8A			+	+	-	+	-	+	+	
S_9	B_1T_9S	+	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	B_1T_9G			+	+	-	+	-	+	+	
	B_1T_9A			+	+	-	+	-	+	+	
S_{10}	$B_1T_{10}S$	+	Rod	+	+	-	+	-	+	+	*Eshcherichia coli*
	$B_1T_{10}G$			+	+	-	+	-	+	+	
	$B_1T_{10}A$			+	+	-	+	-	+	+	

Key: + = positive, - = negative, S_1-S_{10} = Culture numbers, B1= Gombe main Market, T_1 T_{10} =Tilapia fish, C_1C_{10}= Catfish, S = Skin, G = Gut, A = Gill, Oxi = Oxidase, Cat=Catalase, Coa=Coagulase, , Ind = Indole, Cit = Citrate, Man = Mannitol

This indicates that fresh fish with these bacterial pathogens must have been contaminated through improper handling during post-harvest. Similarly, poor handling may led to contaminating fresh fish with man natural flora such as *Staphylococcus aureus*, *Salmonella typhi* [5, 15] and *Eshcherichia coli* which both grow well at 31–37 °C [4]. *Staphylococcus aureus* and *Eshcherichia coli* from the skin of *Clarias gariepinus* indicate improper management and have been implicated in fish-borne diseases of humans [16]. [10], studied mudfish' skin, gut and gill for presence of microbes leading to the identification of *Clarias gariepinus*, *Staphylococcus aureus Eshcherichia coli* and *Pseudomonas fluorescens*. This is similar to the present study findings were *Staphylococcus aureus* and *Eshcherichia coli* was isolated

The results from this study suggest that the microbiological quality of the fish examine is unacceptable and pose a potential risk to public health [10]. The diversity of potential pathogens from the samples is of concern particularly at a time when many in our communities immunologically compromised as a result of various illnesses. Incidence of the faecal coliforms in fish demonstrates the level of pollution of their environment because coliforms are non-indigenous pathogens that contaminate fish.

The isolation of *Staphylococcu aureus* and *Eshcherichia coli* indicates faecal and environmental pollution [12]. Coliforms such as *Eshcherichia coli* are usually present where there has been faecal contamination from warm blooded animals [16]. In contrast, [14] suggested that *Staphylococcu aureus* seldom occurs as natural microflora of fish and shellfish, although, its main habitat is humans and animals. The organism *Eshcherichia coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers it is an indicator of mishandling. *Eshcherichia coli* is the only species in the coliform group that is found in the human intestinal tract and the other warm blooded animals as a commensal and is subsequently excreted in large quantities in faeces. The fish in this study harbored human disease causing microorganisms, that cause such as food poisoning, diarrhea, typhoid fever [14]. Further, such pathogens are most likely to cause food-borne diseases, hence, stringent regulations and monitoring activities coupled with food safety training of suppliers (fishermen and traders) and ultimately the consumers, as well, aspects of Good Hygiene Practice (GHP) and Good Manufacturing Practice (GMP) is needed.

4. Conclusion

Conclusively, the fish sold in Gombe main market contain low microbiological quality compared to that of Dadin-kowa. Poor hygiene practices occurred during marketing help to make the fish highly contaminated. The human bacterial pathogens such as *Eshcherichia coli* and *Staphylococcus aureus* were isolated from the two fish species (Tilapia and cat fish). The presence of large population of these bacterial pathogens indicates high levels of faecal contamination from the

environment. During marketing, it is advisably that fish should not be put on the ground or open to air and dust, but always stored inside container with cover. During transportation, a safety measure such as refrigerated truck should be used to protect fish from potential contamination and damage instead of Lorries. A preservative method like chilling is important during selling the fish to stop or delay the microbial spoilage of fish. The environmental health and hygiene of markets is important to have safe and good foods while, at home, the consumers must wash the fish adequately prior to cooking.

Conflict of Interest

The authors declare that they have no conflict of interest.

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