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Identification of bacterial flora for the Nile Tilapia (*Oreochromis niloticus*) fillet product sold in fish markets of Jimma, Ethiopia

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Abstract

A research exercise was undertaken on isolation and identification of bacteria associated with packed fillets of Nile Tilapia fish *(Oreochromis niloticus)* sold at Jimma town, Oromia, southwest of Ethiopia since March, 2015 to June, 2015. Tissue sections (fillets) of randomly selected Nile Tilapiafishes were studied upon. A serial dilution up to 10^4 was carriedout, and studied on nutrient agar. A total of four bacterial families were isolated and identified as *Staphylococci, Enterobacteriaceae,* Coliform, and aerobic mesophilic bacteria. Out of 73 isolates, 24 isolates were found as Gram positive and 49 were Gram negative; 22 cocci while 51 isolates were identified as rod shaped; 10 isolates were found to have endospore whereas 63 of isolates were non-spore forming bacteria; and 67 of isolates were catalase positive with only 6 catalase negative isolates. The number of colony counted from the plate indicated that *Enterobacteriaceae* were identified as the highest load ($8.92x10^6$CFU/ml), while *Staphylococci* the least ($2.755x10^6$CFU/ml). The mean bacterial load of isolates ($6.1x10^6$ CFU/ml) was found to be markedly higher than the recommended public health and standard value (5.0×10^6 CFU/ml) which has been adopted by most countries.

Keywords: Isolation, Identification, Pathogenic bacteria

Introduction

Fish is an important source of food for many people and subsidizes about 60% of the world's supply of protein (Abisoye*et al.*, 2011).60% of the developing countries derive 30% of their annual protein from fish (Abisoye *et al.*, 2011). It has been thought as human's most important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population. Fish constitutes the cheapest source of animal protein in Africa (Clucas and Ward, 1996). It is one of the main food components of humans for many centuries and still constitutes an important part of the diet of many countries. The advantage of fish as a food resulted from its easy digestibility and high nutritional value.

Fish are susceptible to a large variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Lipp.E.K. 1997). The kind of microorganisms found associated with a

particular fish depends on the water it was found (Thatcher and Clark, 1973; Claucas and Ward, 1996).It is thought that the microbiological diversity of packed fish muscle depends on the fishing grounds and environmental factors around it, the type of micro-organisms that are found associated with particular fish depends on its habitat. Kvenberg, (1991) and Rodricks, (1991) classified the bacterial pathogens associated with fish as indigenous and non-indigenous.

The non-indigenous bacteriainclude *Escherichia coli, Clostridium botulinum, Shigelladynteriae, Staphylococcus aureus, Listerimonocytogens*and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish's habitat like*Vibrio-species* and *Aeromounaus*species. The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, poor water quality, overstocking, which allow opportunistic bacterial infections to prevail (Petronillah R. Sichewo*et al.*,2013).

Pathogenic bacteria associated with fish and shellfish comprise of *Mycobacterium, Streptococcus spp., Vibrio spp., Aeromounaus spp., Salmonella spp*. Andothers (Lipp.E.K.*et al.,* 1997).There are bacterial species that are facultative pathogenic for both fish and humans and may be isolated from fish without apparent symptoms of the disease. Transmission of the pathogens can be through food or the handling of the fish (Mhango *et al*., 2010). There have been great economic losses reported due to food borne illness such as dysentery and diarrhea resulting from consumption of contaminated fish (Mhango *et al*., 2010).

Tilapia is the common name broadly applied to a group of cichlid fishes native to Africa, the Mediterranean, and the Middle East. Tilapia are some of the oldest cultured fishes, as depicted in line drawings originated in Egyptian tombs that date back to 2000 BC). There are numerous diverse species of Tilapia. Aquaculture producers have developed various breeds or hybrids that grow efficiently to market size and have desirable appearance and flavor characteristics. The approved market name for all varieties is 'Tilapia', and the three primary species in the marketplace are: Nile or Black Tilapia *(Oreochromisniloticus)*, Blue Tilapia (*O. aureus*), and Mozambique or red Tilapia *(O. mossambicus)*. Although the species names imply different colors, the edible fillets or portions are quite similar and extra influenced by growing conditions and feeds than external colors. Tilapia is popular because it is a mild flavored, white-fleshed fish that is available throughout the year at a competitive price. The most popular product form is skinless and boneless fillets ranging in size from 3 to 9 ounces (5 to 7 ounce fillets are the most common)

If these waters have been contaminated with sewage, there is a hazard that enteric organisms from infected people may be present and will be concentrated by nourishing activities of fish (Adams *et al.,* 1999). Also while handling of the commodity, the natural flora of the environment may be contaminated with organisms related with man such as members of *Enterobacteriaceae* and *Staphylococcus aureus* which can grow well at 30-37^{o}C (Miceal *et al.*, 2007).

By monitoring the bacteria contents of packed fish fillets, the quality of fish can be measured since these will affect the storage life and quality of the fishery products**.** As a result of the critical role played by fish production in meeting the protein demand, information on the bacterial quality of fish fillet becomes chiefly important, as they are known to significantly affect yield in fisheries (Hudson *et al.,* 2005). The bacteria are responsible for about 45% loses in fish farms (Kabata, 2008).

The issues of safety and quality in packed fish are placed high due to its exceedingly perishable nature (Huss 1995). Fish flesh

naturally contains very low levels of carbohydrates and these are further depleted during the death struggle of the fish (Adams *et al.,* 1999). This has two important consequences for spoilage.

Firstly, it limits degree of acidification of the tissue so that the ultimate pH of the muscles is 6.2-6.5 (Adams *et al.,* 1999). The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with (Douglas, 2007).Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas, 2007).

Secondly, the absence of carbohydrate means that bacteria present on the fish will immediately resort to using the soluble pool of readily assimilated nitrogenous material producing off-odor. The speed with which a product spoils is related to the initial microbial load on the product: the higher the count, the sooner spoilage occurs (Adams *et al.*, 1999).The fresh water or rivers and lake have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources. (Adams*et al.* 1999).

In various ways, fish could be contaminated by microorganisms. Harmful microorganism could enter aquatic food processing chain due to inadequate process control, poor standards of hygiene and sanitation in processing and post-production contamination during improper handling or storage (Clucas and Ward, 1996).

Currently, pathogenic bacterial contamination of fish and fish product is the alarmingly increasing problem in different parts of Ethiopia which also affects life of many residents in Jimma town in similar ways (Personal communication). Fish quality inspection is not well organized in fish shops of Jimma town probably due to lack of knowledge in packed fish quality management (Personal communication).In addition to this, institutional capacity and inadequate availability of resources further constrain fish product quality checks in Jimma town. Therefore, this work should fill the gap through isolation and identification of bacteria from Nile Tilapia *(Oreochromisniloticus)* fillets and established necessary pre-cautions and protection against pathogenic bacteria.

The microbial association with fish includes safety and the quality for human consumption; chiefly grave when the microorganisms are opportunistic or pathogenic in nature (Mhango *et al*., 2010). The dangers of contracting aquatic food borne diseases by the inhabitants from the nearby communities that are using the contaminated fish from the Jimma town are not studied well. These circumstances prompted this research to investigate the occurrence of any human bacterial pathogens from the fillets of Nile Tilapia *(Oreochromisniloticus)* that is sold in various fish shops of Jimma town. The general objective of this study was to isolate and identify bacterial species from Nile Tilapia *(Oreochromisniloticus)* fillet samples from selected fishshops of Jimma town.

The specific objectives of this study are to ascertain and identify the type of pathogenic bacteria associated with packed Nile Tilapia *(Oreochromisniloticus)*commonly consumed by people in the study site. To isolate and quantify the human pathogenic bacterial load associated with packed Nile Tilapia *(Oreochromisniloticus)* fillets.

Materials and methods

Description of the Study Area

The study was carried out in Jimma town, located at 353km southwest of Addis Ababa. The geographical location of the town is 741'N latitude, 3650'E longitude, and an average altitude of 1,780m above sea level. The average minimum and maximum temperature of the town is 14^{o}C and 30^{o}C,

respectively with annual rainfall ranges from 1138-1690mm (Alemu*et al.,* 2011). The experiment was conducted in Jimma University main campus of microbiology laboratory and the study covered duration from February, 2015 up to June, 2015.

Sampling technique

A systematic random sampling technique was used to comprehensive the representative of fish shop sellers.

Media Preparation

Nutrient agar was prepared by weighing 28g and dissolved in 1 liter of distilled water. The dissolved nutrient agar was then autoclaved at a temperature of 121°C for 15 minutes. The media was allowed to cool down and poured into sterilized plates. It was then solidified and incubated at temperature of 37°C for 24 hrs (Microbiology Manual).

Sample collection

A total of 4 samples of packed Nile Tilapia *(Oreochromisniloticus)* fillets were purchased from two different fish shops in Jimma town randomly during the early morning hours of the day (between 7:00 and8:00hr local time). Then each fish sample was put into sterile polythene bag or packed in ice boxes which were later transferred to the microbiology laboratory of the Jimma University for isolation, identification and determination of bacterial load from fillet samples (Ibrahim B. U.*et al.*)

Sterilization of materials

All the glass-wares were washed, dried and sterilized in hot air oven at a temperature of 160°C for 1 hr according to the method described by Adibe and Eze (2004). Culture media were then sterilized in an autoclave at a temperature of 121°C for 15 min.

Preparation of stock cultures

Nile Tilapia (*Oreochromisniloticus*) fillet samples were prepared using the method described by Obi and Krakowiak (1983). About 10g of Tilapia (*Oreochromisniloticus*) fillet sample was cut with a sterile knife. The samples were crushed into small pieces in a sterile mortar with about 10 ml sterile water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This was done for the 4 fish samples.

Preparation of serial dilution

Nine milliliters of sterile water was poured aseptically into four tubes each and 1 ml of the original crushed fish sample was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. This procedure continued until the fourth test tube. The crushed sample was therefore diluted from 10^{-1} to 10^{-4} for each fish sample (Ibrahim B. U.*et al.,* 2014).

Inoculation of plates

Duplicate plates of nutrient agar were inoculated with 0.1 ml of the diluted solution using glass spreader technique. All plates were incubated at a temperature of 37°C for 24 hrs before colony enumeration and isolation. The temperature was chosen to differentiate the mesophile which constitute most medically important pathogenic bacteria (Baker and Silverton, 1985.

Enumeration, isolation and identification of Bacteria

Four serial dilutions of the original stock culture were prepared. Each dilution was plated on solidified freshly prepared nutrient agar and spread using sterile glass rod and incubated at 37^{o}C for 24 hours after which

the colonies that developed on the plates were counted. Distinct colonies from each plate were then picked by means of a sterile wire loop and sub cultured onto a freshly prepared nutrient agar medium contained in sterile plates. This was done with a view to obtaining pure culture of the growth. The observed colony growth was counted using Colony counter according to plate count method. Those counts within 30-300 colony forming units (CFU) was reported as total viable count (TVC).The catalase tests and KOH tests were done to identify isolates as contained in Chessbrough (2000) and Cowan and Steel (1999).

Estimation of bacterial load

The bacteria load was estimated as follows:

Aerobic mesophilic bacterial count

From appropriate serial dilution, 0.1ml of the aliquot was plated on plate count Agar (PCA) and incubate at 32^{o}C for 48hrs (Weil *et al.,* 2006).

***Enterobacteriaceae* count**

From appropriate serial dilution, 0.1ml of aliquot was plated on MacConkey agar and incubated at 32^{o}C for 24hrs.Then the pink to red purple colonies were counted as *Enterobacteriaceae* (Spencer *et al.,* 2007).

Coliform count

From appropriate dilution, 0.1ml of the aliquot was plated on pre-solidified Violet Red Bile Agar (VRBA) plates. Then, the plates were incubated at 32^{o}C for 48hrs.After this, purplish red colonies surrounded by reddish zone of precipitated bile were counted as coli forms (Weil *et al.,* 2006).

Aerobic bacterial spore count

The appropriate serially diluted sample was heat treated in water bath adjusted at 80^{o}C for at least 10 minutes. Thereafter, 0.1ml of the aliquot was plated on pre-dried surface of Nutrient Agar (NA) and incubated at 35^{o}C for 48hrs (Acco *et al.,* 2003).

***Staphylococci* count**

From appropriate dilutions, 0.1 ml of the aliquot was plated onto Mannitol Salt Agar (MSA) and incubate at 32^{o}C for 48hrs.Then the golden yellow showing colony on MSA was repeatedly purified using nutrient broth and pure culture of isolates will be observed microscopically and test with some biochemical (Acco *et al.,*2003).

Microbial analysis

From aerobic mesophilic countable plate, 10 to 15 colonies with distinct morphological differences were randomly picked from plates and aseptically transferred into a test tube containing 5 ml of nutrient broth. Then incubated at 32 ^{o}C for 24hrs.The repeatedly sub-culturing isolates were characterized up to genus level (John, 2012).

Cell morphology

The cell morphology of pure isolates was examined microscopically after Gram staining, and also motility of isolates were checked using motility medium.

Gram staining

A loop full of young culture of fish isolates was dropped and thinned on slides to form smear, and staining with crystal violet for 1min.Thereafter, it was washed gently with tap water for 2 to 3s, then, flooded with iodine solution for 1min before washed with tap water, and also decolorized with 95% ethanol for 15s.Then, the slide was counter stained with safranin for 1min and washed with tap water. The cell morphology of the isolates was observed under microscope using oil immersion objective (Gram, 1884).

Endospore staining

A loopful of pure colony was transferred and thinned on slides. After the smear formation, the slides were flooded with malachite green

and allowed staying on the boiling water for 5 min. Following to washing with water, the smear was counter stained with safranin for 30s.Next to washing with water, the stained culture was observed under the microscope using oil immersion objective (Kim, 2003).

Biochemical test

KOH test

Two drops of 3% of KOH solution was placed on clean slide. A colony was aseptically picked up from the surface of nutrient agar using an inoculating loop and by stirring in the KOH solution for 10s to 2min. The inoculating loop was raised slowly from the mass when the KOH solution became viscous, the thread of slime follow the loop for 0.5 to 2cm or more in gram negative bacteria. While addition of KOH there was no slime, but a watery suspension that does not follow the loop, the isolate was considered as Gram positive bacteria (Gregerson, 1978).

Catalase test

From 24hrs old culture of nutrient broth, a loopful of culture was inoculated into the slant of nutrient agar and incubated at 32 for 24hrs.After incubation; the tubes were flooded with 1ml of 3% catalase and the formation of gas bubbles was observed. The occurrence of gas bubbles were taken as positive for Catalase test (MacFaddin, 1980).

Statistical analyses

The isolated and identified bacteria from fish samples were analyzed using simple descriptive statistical methods and the results were described in the form of tables.

Ethical consideration

Informed consent was obtained from Ethical committee of Natural and computational science and Research review of Jimma University. Sampling of fish was carried out with full permission of the of fish shop owners. Before each sampling, the study objectives was clearly explained to the owners and workers of fish shops that the aim of the study was not be neither to evaluate the performance of the individual nor to blame anyone for weakness, but to gather information that might lead to eventual improvement in production quality. By respecting their beliefs and culture the respondent were informed about the objective of the study so as to collect fish samples easily for bacterial analysis.

Results

The mean difference of microbial count from fish samples was summarized in table 1. Accordingly, Aerobic mesophilic count was found $6.1x10^6$ CFU/ml, whereas count of *Staphylococci* was $2.755x10^6$ CFU/ml, and that of *Enterobacteriaceae* was $8.92x10^6$ CFU/ml, Coliform bacterial count was $6.79x10^6$ CFU/ml.

Morphology and biochemical tests

The morphology and Biochemical tests resulted from 4 samples obtained from73 isolates were summarized in Table 2, 3, 4 and 5 below respectively. The Pink to red purple colonies of bacteria grown on MacConkey agar were identified as *Enterobacteriaceae*, whereas Purplish red colonies surrounded by reddish zone of precipitated bile were coli form bacteria. Then the golden yellow showing colony on MSA were isolated and identified as staphylococci bacteria.

The present study revealed that, out of 73 isolates 51 had rod shape while 22were cocci (Table 2), and 10 isolates had endospore whereas 63 isolates had no endospore (Table 3), 24 of the isolates were gram positive while 49 were gram negatives (Table 4).

Biochemically, 67 isolates showed catalase positive whereas only 6 isolates showed catalase negative(Table 5). From this context, majorities of isolates were rod shaped, non-spore forming, catalase positive, KOH positive (gram negative) bacteria (Table 5).

Table 1: Mean Bacterial counts of four packed *Oreochromisniloticus* fish filets samples sold in two shops in Jimma town.

Bacterial counts	DF	Mean Number of bacterial colonies		Mean of A and B colony	CFU/ml	Mean of CFU/ml
		Sample A	Sample B			
Staphylococci count	10^{-3}	0	122	61	$6.1x10^5$	$2.755x10^6$
	10^{-4}	0	98	49	$4.9x10^6$	
Coliform bacterial count	10^{-3}	173	103	138	$1.38x10^6$	$6.79x10^6$
	10^{-4}	168	76	122	$1.22x10^7$	
Aerobic mesophilic bacterial count	10^{-3}	145	135	140	$1.40x10^6$	$6.1x10^6$
	10^{-4}	119	97	108	$1.08x10^7$	
Enterobacteriaceae count	10^{-3}	195	173	184	$1.84x10^6$	$8.92x10^6$
	10^{-4}	172	148	160	$1.60x10^7$	
Total	-	972	952	962		$6.1x10^6$

Note: DF-Dilution factor, Sample A was from Hawetu and Sample B was from Kochi.

Table 2: Shapes of 73 Bacterial isolates from packed Nile Tilapia (*O. niloticus*) fish filets sold in Jimma town.

Morphological features (shapes) of isolates from packed *O. niloticus* fish fillets		
		Number of isolates
Cell shape of isolated bacteria	Cocci	22
	Rod	51

Table 3: Endospore of 73 Bacterial isolates from packed Nile Tilapia (*O. niloticus*) fish filets sold in Jimma town.

Morphological features (Endospore) of isolates from packed O. niloticus fish fillets		
		Number of isolates
Endospore	-	63
	+	10

Table 4: Gram reaction of 73 bacterial isolates from packed Nile Tilapia (*O. niloticus*) fish filets sold in Jimma town.

Morphological features (Gram's stain) of isolates from packed *O. niloticus* fish fillets		
	Negative/positive	Number of isolates
Gram reaction	-	**49**
	+	**24**

Table 5: Biochemical tests of the 73 bacterial isolates from Jimma town.

Biochemical tests		Number of isolates
Catalase test	+	67
	-	6
KOH test	+	49
	-	24

Discussion

The mean difference of microbial count obtained from *Oreochromisniloticus* fish fillets samples purchased from Hawetu and Kochi Kebele were indicated as follows. The Aerobic mesophilic bacterial count was found to $6.1x10^6$ CFU/ml, whereas, the mean of the colony per milliliter of *Enterobacteriaceae* count obtained from fish fillets were indicated numerically as $8.92x10^6$ CFU/ml. The Coliform bacterial count isolated from the packed fillets of Nile Tilapia was $6.79x10^6$ CFU/ml.

The presence of faecal coliforms in fish demonstrates the level of pollution of their environment because coliforms are not the normal habitat of fish. Clucas and Ward (1996) recorded that *S. auerus* seldom, if ever, occurs as natural micro-flora of fish and shellfish; its main habitat is humans and animals and is found mostly in the nose, throat and skin of healthy individuals. This suggests that packed fish with this pathogen post-harvest must have been contaminated through handling. Contamination of packed fillet of fish with *E. coli* also suggested that one or more of enteric pathogens have gained access to the fish. This organism (*E. coli*) is particularly useful as an indicator of contamination when appeared in small numbers or as an indicator of mishandling when appeared in large numbers.

The bacterial load of packed Nile Tilapia (*Oreochromis niloticus*) fish fillets from selected shops in Jimma town were set ordered according to the mean value of bacterial colonies isolated and identified from packed fish fillets. The current result elicited that, the highest bacterial load and the lowest bacterial load isolated and identified were *Enterobacteriaceae* and *Staphylococci* respectively. The *Enterobacteriaceae* demonstrated as the indicator of pathogens whereas the existence of aerobic bacterial spore count revealed the contamination of packed fish fillets with spore forming bacteria like Bacillus in adverse manner (Spencer *et al.*, 2007).

Even though the overall microbial load per milliliter of fish samples appeared to be in the acceptable ranges, one should also think of possibility of pathogen presence as detection of bacteria such as *Enterobacteriaceae* reveal poor quality(if not safety) of packed fish fillets probably during catching, transporting, packing, processing, handling or storage. Such poor processing and handling storage probably had significant effects on shelf life of the packed fish fillets of Nile Tilapia *(Oreochromis niloticus).*Assessments of the levels of *Enterobacteriaceae* in aquatic foods allow an estimation of their general bacteriological condition ('indicator' function) and to a certain extent the risk of presence of pathogenic enteric organisms ('index' function) (Mossel, 1982).The mean viable count of the organisms from the study was found to be higher than the Nigerian Agency for Drug Administration and Control (NAFDAC) recommended official public health standard of between 5.0 x 105 and 1.0 x 106 CFU g-1. The mean viable count although cannot be taken as an absolute figure.

This is probably due to the number and type of bacteria found on fillets of packed fish is dependent on many factors, of which, source of the fish contributes the major factor and supports Thatcher and Clark (1973) earlier report which stated that the kind and number of microorganisms found on the fillets of packed fish is dependent on the source of the fish, additional contamination introduced in the fishing boat, freezing temperature during storage, severity of packing process with respect to lethality to microorganisms and contamination by handlers and market sellers. Brooks *et al*., (2004) however, concluded that one of the sources of infection is contaminated aquatic food which is probably similar cause of infection in Jimma town.

The identification of isolated bacteria was carried out through Gram's reaction and biochemical tests (KOH and Catalase test).As indicated from the table: 4above, the Gram's reactions of the 73 isolated bacteria were categorized in to two as Gram positive and Gram negative bacteria. Out of 73 isolates, 24 isolates were Gram positive while 49 isolates were Gram negative bacteria. But it must considered that the Gram stain reaction is not always a true indicative of the organism's cell wall structure

Poorly controlled decolorizing can obviously be a source of misleading results, but even with adequate technique some organisms are problematic (Fisheries Service, 2011). For example, some strains of *Bacillus* or *Clostridium* consistently stain Gram negative even though they have a Gram positive type cell wall (Gram, 1884). The KOH test relies on the differential resistance to 3% potassium hydroxide between Gram positive and negative cells, where a portion of a colony is mixed with a small volume of 3% KOH on a glass slide for no more than 60 seconds.

If the cells lyses, the liberated cellular DNA makes the mixture viscous or "stringy." The positive string test indicates a Gram negative organism (Fisheries Service, 2011).

Biochemically, the isolates were tested with catalase to distinguish *Staphylococci* from *Streptococci* (MacFaddin, 1980). Accordingly, from the total of bacterial isolates, 67 isolates were catalase positive whereas only 6 bacterial isolates were catalase negative. The result elicited that majority of the isolated bacteria were KOH positive and catalase positive. The catalase test was used to differentiate staphylococci (catalase-positive) from streptococci (catalase-negative) (MacFaddin, 1980).

The enzyme, catalase is produced by bacteria that respire using oxygen, and protects them from the toxic by-products of oxygen metabolism. Catalase-positive bacteria include strict aerobes as well as facultative anaerobes, although they all have the ability to respire using oxygen as a terminal electron acceptor (MacFaddin, 1980). Catalase-negative bacteria may be anaerobes, or they may be facultative anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (i.e. *Streptococci*) (MacFaddin, 1980).

Generally, higher *Enterobacteriaceae* count was probably due to the poor general sanitation of the seller and lack of clean environment because most of the bacteria thrive under unhygenic environment. On the other hand, the presence of *Staphylococci* could be an indicator of poor handling and contact with bare hand. The *Enterobacteriaceae* presented the indicator of pathogens whereas the existence of aerobic bacterial spore count revealed the contamination of packed fish fillets with spore forming bacteria like *Bacillus* in adverse manner.

It is therefore necessary that close supervision of packed fish fillets should be carried out by relevant authorities to reduce the risk of this aquatic food. Therefore, proper processing of packed fillets of Nile Tilapia fish sample should be carried out before consumption.

Conclusion

The present study revealed that all fish fillet samples taken from the packed Nile Tilapia *(Oreochromis niloticus)* fish sold in Jimma town were contaminated with different bacterial species such as *Enterobacteriaceae*, coliform bacteria, aerobic mesophilic, and *Staphylococci*. The result clearly indicated that inadequate hygienic condition during catching, transporting, storing (packing) of the fish and calls for the close supervision, regulation and awareness creation by the concerned bodies in order to minimize the potential risks of these pathogenic bacteria.

From the result obtained and conclusion made above, the following points were raised as recommendation. Catching, transporting, and packing of Nile Tilapia *(Oreochromis niloticus)* fish fillets using the appropriate tools and crafts are very critical. Creating awareness among fishermen, transporters, sellers and consumers is necessary by the concerned officials to pay attention to ensure fish fillets quality issues. Enhancing fishermen and sellers to follow strict hygiene procedures during catching, transporting, packing, and selling of fish fillets is needed. Awareness for users about sources of microbial contaminations in fish fillets (equipments, human, water, dust).

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Abbreviations and Acronyms

CFU - Colony Forming Units
FAO - Food and Agricultural Organization
FDF - Federal Department of Fisheries
MSA - Mannitol Salt Agar
NA - Nutrient Agar
PCA - Plate Count Agar
TVC - Total Viable Count
VRBA - Violet Red Bile Agar
WHO - World Health Organization