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Determination of microbial contamination in Parangipettai coastal water fish during handling

S.Velmurugan[1*], Shani T.John[1], Sheta Alemu[2] and D. Senthil Nagaraj[2]

[1] Department of Biology, Madawalabu University, Post Box No: 247, Bale Zone, Robe, Ethiopia.

[2]Department of Chemistry, Madawalabu University, Post Box No: 247, Bale Zone, Robe, Ethiopia.

Abstract

The sea food is considered highly nutritious and less harmful when compared to other red meats. In the present study, the total heterotrophic bacterial population density was recorded in four different site fish samples. The microbial load of freshly caught fish was low, ranged from $1.21x10^3$ to $1.42x10^3$, followed by fish after two hours $7.62x10^3$ to $8.83x10^3$, fish at landing center were $2.0x10^4$ to $3.22x10^4$ and fish at market ranged from $4.91x10^4$ to $6.07x10^4$. The total microbial load was increased along with pathogenic microbes. In this study totally 25 strains were isolated from fish samples, of these isolates 9 genera were identified based on the biochemical characters. The names of the bacterial genera were *Vibrio* (17.5%), *Pseudomonas* (22.5%), *Shigella* (5.75%), *Bacillus* (15.25%), *and Escherichia coli* (15.5%), *Klebsiella* (8.5%), *Staphylococcus* (7%), *Corynebacterium* (5%) and *Salmonella* (3%).

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Corresponding address : S.Velmurugan1
Department of Biology, Madawalabu University, Bale Zone, Ethiopia
velkas.cas@gmail.com

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Introduction

Fish and seafood constitute an important food component for a large section of world population (Bark et al., 2011; Sakthivel and Fernando, 2012; Trivedi et al., 2012; Ozcan et al., 2013; Varadharajan et al., 2013). Fishery products can also be a source of various food borne diseases, (Darlington and Stone, 2001). It acts as a vehicle for all important species of food borne pathogens. Environmental conditions play an important role on the pathogens count in fresh fish (Bark et al., 2011). Shellfish may be contaminated with food borne pathogens which are naturally present in aquatic environments, such as Vibrio spp. or derived from sewage contaminate water such as Salmonella (Ali and Hamza, 2004). In the developing world, food borne infection leads to the death of many children, as well as resulting in diarrheal disease which can have long-term effects on children's growth as well as on their physical development and it also heavily affects the healthcare systems (Adak et al., 2005).

According to Clarence et al., (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. The quality of fresh fish and seafood products is rapidly reduced as a consequence of various microbial, biochemical and chemical breakdown processes. The initial quality loss is mainly due to the post mortem autolytic activity and chemical degradation processes, such as lipid oxidation. The rate of quality loss depends directly on the nature of the fish species in question, as well as handling and storage conditions. Analyses of the microbial load and diversity are used to determine the amount of specific spoilage bacteria in a sample as well as the total bacterial count. For marine fish stored aerobically on ice, the bacterial flora is well studied, and dominated by Pseudomonas spp. (Gram and Huss, 1996). Knowledge of spoilage organisms and their specific activity in various fish species at different storage conditions has led to more precise shelf-life predictions and facilitated modeling of spoilage (Gram and Dalgaard, 2002). ¬ The main purpose of this study was to determine and evaluate the microbial contamination of fish *Mugil cephalus* collected from catching point to fish vendors.

Materials and Methods

Sampling Procedure

A total number of 90 samples of fish *Mugil cephalus* were collected from Parangipettai coast, South East coast of India.

Fish from different sites, such as freshly caught fish, after two hour fish, fish at landing centre and fish at local market were collected. After collection, the samples were individually transferred to clean and unused polythene bags and transported to the laboratory in a portable ice chest box. Aseptic procedures were adopted to minimize secondary contamination. Seafood with visible signs of deterioration, injury and disease was discarded.

Bacteriological Analysis

Enumeration of Total Heterotrophic Bacteria (THB)

Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar was used for the selective isolation and culture of Vibrios. Eosin Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (SSA) were used for E.coli and Salmonella, Shigella. MacConkey agar with 0.15 % bile salt, crystal violet and NaCl has been used in accordance with USP/nfxi (1) for the detection, isolation and enumeration of coli forms. Cetrimide agar was used as a selective medium for the isolation of *Pseudomonas aeruginosa*. The plates were used for different samples and the plates were incubated at 37°C for 24-48 hr for evidence of growth. Pure isolates of resulting growth were identified using biochemical methods as described by Jolt et al. (1994).

Results

Total heterotrophic bacterial population

Total heterotrophic bacterial population of the fish from four different sites were analyzed and recorded. The microbial load of freshly caught fish was low, ranged from $1.21x10^3$ to $1.42x10^3$, followed by fish after two hours $7.62x10^3$ to $8.83x10^3$, fish at landing center were $2.0x10^4$ to $3.22x10^4$ and fish at market ranged from $4.91x10^4$ to $6.07x10^4$. The total microbial load was increased along with pathogenic microbes (Table.1).

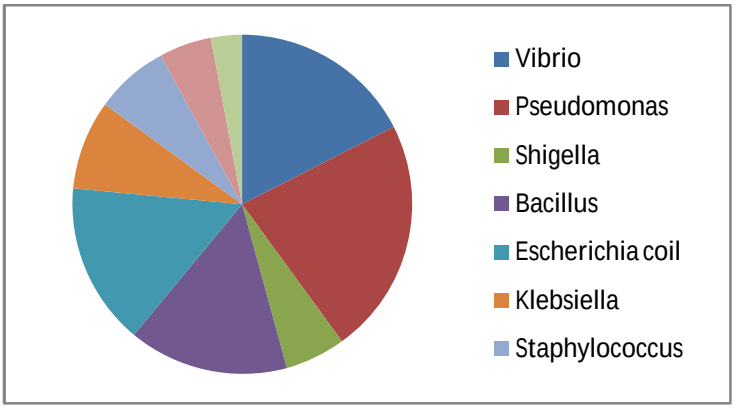
Generic composition of total heterotrophic bacteria

The total number of 25 strains were isolated from four different site fish samples and randomly selected, sub-cultured and identified up to generic level. The total of 9 genera were recorded from the samples such as *Vibrio* (17.5%), *Pseudomonas* (22.5%), *Shigella* (5.75%), *Bacillus* (15.25%), *Escherichia coli* (15.5%), *Klebsiella* (8.5%), *Staphylococcus* (7%), *Corynebacterium* (5%) and *Salmonella* (3%) (Fig.1). The result showed the presence of more number of gram negative genera than the gram positive genera. Totally 6 genera recorded namely *Vibrio, Pseudomonas, Shigella, E. coli, Klebsiella* and *Salmonella* belong to the gram negative group.

Table 1. THB population in four groups of fish samples

Samples	Minimum	Maximum
Freshly caught fish	$1.21x10^3$	$1.42x10^3$
Two hour incubated fish	$7.62x10^3$	$8.83x10^3$
Fish at landing center	$2.0x10^4$	$3.22x10^4$
Fish at market	$4.91x10^4$	$6.07x10^4$

Fig.1. Percentage composition of microorganisms



Discussion

Microbes play an important role in determining the quality of any aquatic environment. In the marine environment, they balance characteristically in different ecosystem in which they live. In this study total bacterial population was recorded from in freshly caught fish was low, ranged from $1.21x10^3$ to $1.42x10^3$, followed by fish after two hours $7.62x10^3$ to $8.83x10^3$, fish at landing center were $2.0x10^4$ to $3.22x10^4$ and fish at market ranged from $4.91x10^4$ to $6.07x10^4$. Prabakaran et al., (2011) reported the total plate count showed maximum in the processing area samples in fish samples ($65x10^4$ CFU.g-1). The Higher microbial counts in some samples may be attributable to handling during harvest or processing.

The total bacteria count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of effecting health conditions of consumers of such contamination fish (WHO, 2007). Montville et al. (2002) have similarly concluded that, during handling and preparation, bacteria may be transferred from contaminated hands of food workers to food and subsequently to other surfaces (including food contact surfaces). In this study total of 9 genera were recorded from the samples such as *Vibrio, Pseudomanas, Shigella, Bacillus, Escherichia coli, Klebsiella, Staphylococcus, Corynebacterium* and *Salmonella*. Venugopal (2002) studied the contamination of fish particularly by pathogens such as *Salmonella sp., Staphylococcus aureus, Campylobacter jejuni, Escherichia coli, Vibrio parahaemolyticus, Yersinia enterocolitica*, and *Listeria monocytogenes*, may occur prior to harvest, during capture, processing, distribution and storage. The contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Empikpe, 2011). During handling and storage, or while transporting quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product, Adebayo-Tayo et al. (2012b). The quality of fish degrades; due to a complex process in which physical, chemical and microbiological form of deterioration is implicated (Adedji, 2012). Huss et al. (2000) have pointed out that some pathogenic bacteria are naturally present in the aquatic (*Clostridium botulinum* type E, pathogenic *Vibrio* sp., *Aeromonas*) and the general environment (*C. botulinum*, type A and B, *Listeria monocytogenes*) and may therefore be found on live or raw fish. More fascinatingly, fish at market and fish at landing center has recorded more number of pathogenic bacterial populations and comparatively very low population density of E. coli and total absence of the other bacteria have been noticed freshly caught fish. The study done by Reij et al. (2004) attributed poor hygiene, particularly deficient or absence of hand washing as the causative mode of transmission and contaminated surfaces has been observed in many cases and unclean, insufficiently or inadequately cleaned equipment have been identified as a source of bacterial contamination in seafood.

Conclusion

The present study suggested that the contamination level was found increased from the catch point to the consumer end because of unhygienic handling and time taken for selling in the market resulted in deterioration. The present investigation concluded that microbial load increased based on the time duration and improper handling of the vendors. To avoid these problems fish should be washed properly with the natural antibacterial compounds like salt, turmeric and tamarind, after washing the fish should be boiled at a maximum temperature. This process of washing and boiling would be helpful in eradicating the pathogens from the sea food before consumption by the human beings.

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