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Biochemical Identification of Bacteria from the Selected Edible Marine Fishes Available in Thrissur, India

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Abstract: The microbial quality of two common edible marine fishes Indian oil sardine (*Sardinella longiceps*; n=14) and pink perch (*Nemipterus japonicas*; n=14) were collected from Chettuvai fish landing Centre, and from Thrissur Shakthan market, and assessed during the period from November 2017 to January 2018. Swabs were taken from skin and flesh using a sterile knife and mortar and incubated for 24 h at 37 °C. Differentiation and characterization of various isolates were conducted based on their growth characteristics on specific culture media. Mac Conkey agar and SS agar were used. The results revealed that the samples were contaminated by nine bacterial species, which includes *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, *Shigella* spp., *Salmonella typhi*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Staphylococcus aureus*. Among the nine bacterial species *Escherichia coli*, *Salmonella typhi* and *Shigella* spp. were found in all the collected samples. The result of this study revealed that raw fish sold in Thrissur fish market has high contamination so the presence of the bacterial species has strongly suggested the urgent need to improve the quality control systems in Thrissur fish market. Since the contamination in these fishes was higher than those fishes sold in Chettuvai fish landing Centre, the contamination is mainly due to the handling and improper processing of the fish. This investigation is mainly focused on the pathogenic microbes of these two edible marine fishes and to create awareness in disease transmission to man.

Keywords: Biochemical, Microbials, *Sardinella longiceps*, *Nemipterus japonicas*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*

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Introduction

Fish is a low fat food, a great source of protein, vitamins and minerals. Fish has become an increasingly important source of protein and other elements necessary for the maintenance of healthy body, and constitute an important food

component for a large section of the world population. It is man's most important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO, 1997). Fish allows for

protein improved nutrition as it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe *et al.*, 2011). Fish and seafood products constitute an important food commodity in the international trade due to its ever-increasing consumption demand.

However, fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Lipp and Rose, 1997). The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient or there are other stressors, like poor water quality, overstocking which allow opportunistic bacterial infections to prevail. A number of reports suggested that the consumption of the microbiologically spoiled seafood might be responsible for food-borne diseases like diarrhea, salmonellosis, shigellosis, cholera and even some neurological diseases by an array of viruses, bacteria, fungi, and parasites (Wallace *et al.*, 1999; WHO, 2012). *Salmonella* spp. is responsible for the most of the food-borne diseases or gastroenteritis characterized by diarrhea, abdominal cramp, vomiting, nausea, and fever.

The present study was performed to investigate the microbiological quality of the edible marine fishes for raising the food safety concern and promoting international trade. In view of the various ways fishes could be contaminated with microorganisms, the present study was therefore aimed at finding out if fish consumed in Thrissur district is contaminated with bacterial pathogens and if so, to identify the major bacterial contaminants.

Materials and Methods

Study area:

The study covered Chettuvai and Shakthan fish market in Thrissur, India. Chettuvai is one of the important fishing Centre in Thrissur district, with rigorous activity. The area is located at just 1.5 km west of the NH 47 and 8 km south of Chavakkad in

Engandiyoor village. Chettuvai fish landing Centre is the place where different types of fresh fish commodities were accumulated from different sources of water bodies. These fishes were transferred from here to local markets, including Thrissur Shakthan market, via different intermediaries and channels. Fresh fish samples were collected from Chettuvai as control, and samples collected from Thrissur market were examined for bacterial contamination through handling, transport, processing etc.

Fish sample collection and preparation:

Between November 2017 and January 2018, 7 samples of each selected edible fishes were collected from Chettuvai fish landing Centre and Thrissur Shakthan fish market. A total of 28 fish samples (7 oil sardine and 7 pink perch from Chettuvai, 7 sardine and 7 pink perch from Thrissur market) were purchased from both areas. The fish samples were collected in a clean polyethylene bag and labeled with date and place of collection. Then the samples were transferred immediately to the laboratory for further analysis.

Isolation of bacterial species from the fish:

About 2 g of fleshy part of trunk region of the fish were cut with a sterile knife and the cut samples were crushed into small pieces in a sterile mortar. Also the mucus was extracted from the region of mouth and both extracts were mixed in 10 ml of sterile distilled water, mixed gently for several times. This was done for 28 fish samples. Exactly 1 ml of fish part samples were introduced to the surface of already prepared Mac Conkey agar and SS agar medium. Replicate plates for each preparation were incubated at 37 °C for 24 h.

Characterization of bacterial isolates:

After 24 h of incubation, the bacterial populations were counted. The morphological characteristics of the isolates were examined. Distinct colonies developing on the culture plates were observed for their pigmentation, margin, elevation and opacity. The bacterial isolates were identified and classified using a combination of biochemical methods (Indole test, Catalase test, Urease test,

Table 1: Bacteria isolated from the flesh of *Sardinella longiceps*

Biochemical Tests	A	B	C	D	E	F	G
Gram staining	-	-	-	-	-	-	-
Motility	+	+	+	-	+	+	-
Indole production	+	-	-	-	-	-	-
MR	+	-	-	+	-	-	-
VP	-	+	-	-	+	+	+
Citrate	-	+	+	-	-	+	+
Oxidase	-	-	+	-	-	-	-
Urease	-	-	-	-	-	-	+
Catalase	+	+	+	+	+	+	+
Lactose	+	-	-	-	-	+	+
H ₂ S	-	-	+	-	+	-	-

A: *Escherichia coli*; B: *Aerobacter aerogenes*; C: *Pseudomonas aeruginosa*; D: *Shigella* spp. E: *Salmonella typhi*; F: *Enterobacter aerogenes* G: *Klebsiella pneumoniae*

Motility test, MR VP test, Citrate test, Lactose test, H₂S test and Oxidase test). Streaking is repeatedly done for the isolation of pure culture.

Results and Discussion

The fish are highly perishable and prone to vast variations in quality, due to improper processing, handling, storage etc. they can also function as carriers of several microbial and other health hazards. The diversity of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. The result of the study revealed that the edible marine fishes were contaminated with pathogenic bacteria.

Tables 1 to 4 show the gram staining and biochemical test of pure isolates of bacterial species. It showed that only 9 bacterial species were obtained from the whole total count.

The bacteria identified in fish samples were *Escherichia coli*, *Aerobacter aerogenes*,

Pseudomonas aeruginosa, *Shigella* spp., *Salmonella typhi*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Staphylococcus aureus*. The members of the family Enterobacteriaceae, such as *Escherichia coli*, *Shigella* spp. and *Salmonella typhi* were common bacterial isolates. *Escherichia coli* isolated from both control and tested samples. *Escherichia coli* contamination in the tested samples was higher in the skin compared to flesh. The occurrence of these pathogenic bacteria in fish may influence human health by inducing diseases and cause abdominal pain, acute gastroenteritis, bloody/mucoid diarrhea, nausea, vomiting and fever. *Escherichia 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. In this study, *Pseudomonas aeruginosa* isolated from the collected fish samples is of high importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism.

Table 2: Bacteria isolated from the skin of *Sardinella longiceps*

Biochemical Tests	A	B	C	D	E	F	G
Gram staining	-	-	-	-	+	-	-
Motility	+	-	+	+	-	+	-
Indole production	+	-	-	-	-	-	-
MR	+	+	+	-	-	-	-
VP	-	-	-	-	+	+	+
Citrate	-	-	-	+	-	+	+
Oxidase	-	-	-	+	-	-	-
Urease	-	-	-	-	-	-	+
Catalase	+	+	+	+	-	+	+
Lactose	+	-	-	-	+	+	+
H ₂ S	-	-	+	-	-	-	-

A: *Escherichia coli*; B: *Shigella* spp; C: *Salmonella typhi*; D: *Pseudomonas aeruginosa*; E: *Enterococcus faecalis*; F: *Enterobacter aerogenes*; G: *Klebsiella pneumoniae*

Table 3: Bacteria isolated from the flesh of *Nemipterus japonicus*

Biochemical Tests	A	B	C	D	E
Gram staining	-	-	-	-	+
Motility	+	+	+	-	-
Indole production	+	+	-	-	-
MR	+	+	-	+	+
VP	-	-	+	-	+
Citrate	-	+	-	-	+
Oxidase	-	-	-	-	-
Urease	-	+	-	-	+
Catalase	+	+	+	+	+
Lactose	+	-	-	-	+
H ₂ S	-	+	+	-	-

A: *Escherichia coli*; B: *Proteus vulgaris*; C: *Salmonella typhi*; D: *Shigella* spp; E: *Staphylococcus aureus*

The presence of *Staphylococcus aureus* to the contamination of the fish samples can be attributed to person handling the fish. *Staphylococcus aureus* encountered in this study are known enterotoxin producing agent and a microorganism, which is poisonous (Eze *et al.*, 2011).

In this study isolation of pathogenic Enterobacteriaceae, such as *Escherichia coli*, *Shigella* spp., *Salmonella typhi* from the collected samples indicated public health hazards and concern. The presence of *Salmonella typhi* in the fish samples was in conformity with the result obtained by Petronillah *et al.* (2013). The isolation

of *Klebsiella pneumonia*, *Proteus vulgaris* and *Shigella* spp. from the fish samples indicated fecal and environment pollution and this support the findings of Oskar *et al.* (2014). The occurrence of pathogenic bacteria, especially *Staphylococcus aureus* is in concurrence with Moshood and Tengku (2012). The result of this study revealed that raw fish sold in Thrissur fish market has high contamination so the presence of the bacterial species has strongly suggested the urgent need to improve the quality control systems in Thrissur fish market. Since the contamination in these fishes were higher than those fishes sold in Chettuvai fish landing Centre, the contamination is

Table 4: Bacteria isolated from the skin of *Nemipterus japonicas*

Biochemical Tests	A	B	C	D	E	F
Gram staining	-	-	-	+	-	-
Motility	+	-	+	-	+	-
Indole production	+	-	-	-	-	-
MR	+	-	-	+	-	+
VP	-	+	-	+	+	-
Citrate	-	+	+	+	-	-
Oxidase	-	-	+	-	-	-
Urease	-	+	-	+	-	-
Catalase	+	+	+	+	+	+
Lactose	+	+	-	+	-	-
H ₂ S	-	-	+	-	+	-

A: *Escherichia coli*; B: *Klebsiella pneumonia*; C: *Pseudomonas aeruginosa*; D: *Staphylococcus aureus*;
E: *Salmonella typhi*; F: *Shigella* spp

mainly due to the handling and improper processing of the fish.

Conclusion

It can be concluded that fish from the present study area should be processed with properly treated pathogen-free water and ice, finally, should be maintained at good storage condition. Fish that have shown signs of spoilage should not be sold to the public. Since these microorganisms could contaminate fish and therefore a source of food poisoning; harvesting, handling and cooking of fish should be done properly so as to reduce the bacterial load. Adequate treatment methods before consumption of fish are highly imperative. In this case, eating of raw or half boiled should be discouraged to eliminate zoonotic infections from fish in this area. Coordinated efforts from government sector and private industry together with federal agencies are urgently needed in this context. There is a need for routine surveillance systems using pathogen-specific techniques to avoid any future outbreaks.

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