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An investigation on microbial quality of salted and Sun dried fishes from Pulicat lake fishermen villages

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Abstract
The present study has executed to investigate the microbial quality of the salted and sundried fishes around the Pulicat dry fish market. There were four varieties chosen for this study. The varieties of dry fishes were anchovy (*Stolephorous commersonni*), sardine (*Sardinella longiceps*), mackerel (*Rostrelliger kanagurta*) and silver bellies (*Leiognathus* sp.). The bacterial and fungal infestation was found in the dried fishes. The bacterial strains were total heterotrophic bacteria, *Escherichia coli, Vibrio cholera, V. parahaemolyticus, Salmonella* sp., and *Shigella sp.* The maximum total heterotrophic bacterial count was observed in fish silver bellies (5.2×10^4) followed by anchovy (4.8×10^4), mackeral (4.1×10^4) and sardine (3.9×10^4) respectively. Whereas checking the *Escherichia coli* load on the maximum was observed in mackeral (33) followed by anchovy (27), silver bellies (26) and sardine (21) respectively. The fungal infestations were *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicillium* sp. and *Verticilium* sp. This study revealed that, the quality of processed dried fishes were found with microbiological infestation of different contaminants. Such products should be properly cooked before consumption. Consumers should be continuously sensitized to raise awareness of the existence of such microbes in order to encourage adequate cooking of fish prior to consumption.

Keywords: microbial contamination, sundried fishes, salted fishes, Pulicat Lake

Introduction
Human have been eating sea food since the beginning of recorded history. Fish was the most reliable protein food. Dried fish have relatively low contribution both in terms and quality and value in price. Most of the fishes among by catch are dried in the sun after salting. Although it is a common practice to salt the fish before drying certain varieties of *Anchoviella* are dried without salting. Highly salted fish retards microbial growth. For low salt product halophilic or holotolorent populations consisting of gram negative organism will prevail. Pathogen growth in the finished product as a result of inadequate drying of fishery products can cause consumer illness. Dried products are usually considered shelf stable and are, therefore, often stored and distributed unrefrigerated (Nair, 2003) [4]. Water is the measure of the amount of water in a food that is available for the growth of microorganisms, including pathogens. A water activity of 0.85 or below will prevent the growth and toxin production of pathogens, including *Staphylococcus aureus* and *Clostridium botulinum*.
Poor hygiene condition is because they dry fish are placed on mat, sandy substratum or hung in raised rack/ pole. During handling fish gets contaminated with various types of bacteria if the time temperature condition favours organism grows and multiply and lead to spoilage. Human health on consumption of such fish is dangerous and it can lead to food poisoning and problem to public health. Hygiene and sanitation, therefore plays a vital role in fish handling. In this perspectives the study has planned to isolate and enumerate the bacteria and fungi from four commercially important fishes *viz*, anchovy (*Stolephorous commersonni*), sardine (*Sardinella longiceps*), mackerel (*Rostrelliger kanagurta*) and silver bellies (*Leiognathus* sp.).

Materials and methods
Four dry fish samples were taken for this present study. The dry fishes were sardine (*Sardinella longiceps*) (Fig.1), mackerel (*Rostrelliger kanagurta*) (Fig.2), anchovy (*Stolephorous commersonni*) (Fig.3) and silver bellies (*Leiognathus* sp) (Fig.4).

Fig 1: Sample A (*Sardinella longiceps*)

Fig 2: Sample B (*Rastrelliger kanagurta*)

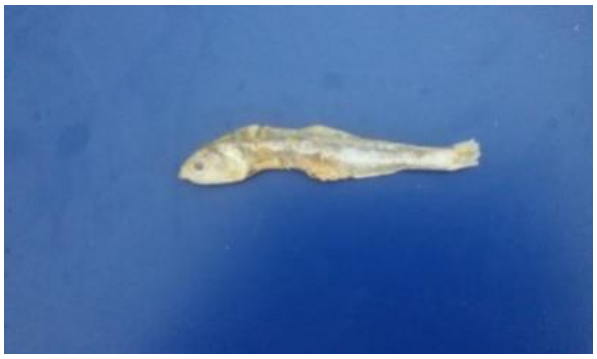

Fig 3: Sample C (*Stolepherus commersonni*)

Fig 4: Sample D (*Leiognathus* sp.)

Enumeration of Total Heterotrophic Bacteria (THB)

For isolation of Total Heterotrophic Bacteria (THB) pour plate method was followed.

The total count was followed by using the formula.

$$THB = \frac{\text{Total no. of colonies}}{\text{Volume of sample}} \text{ X dilution factor CFU/g}$$

Isolation of *Escherchia coli, Vibrio* sp., *Salmonella* sp. and *Shigella* sp.

10g of homogenised fish samples were suspended in 90ml sterile water blank, from this three different dilution such as 0.1ml, 1ml and 10ml are added to three separate series of sterile lauryl tryptose broth (*E.coli*), sterile alkaline peptone water broth (*Vibrio* sp.), sterile selenite cysteine broth (*Salmonella* sp. and *Shigella* sp.) used respectively.

Isolation of Fungi

For isolation of fungi spread and pour plate method was followed. 10g of homogenised fish samples were suspended in 90ml sterile water blank and serially diluted up to 10^{-4}. From the dilution 10^{-3} and 10^{-4} 1ml of sample was taken and spreaded and poured in to sterile potato dextrose agar (PDA) plates and incubated at room temperature for 24 hrs. After incubation period the morphologically different colonies were counted.

Results

Bacteria

A total of four different fish samples were collected from local fish markets at Pulicat lake. For isolation of total heterotrophic bacteria (THB) spread plate method was followed using nutrient agar medium, for the isolation of total coliforms, *E. coli*, *Vibrio* spp., *Salmonella* sp. and *Shigella* sp. MPN technique was followed using EMB agar, TCBS agar and SS agar respectively.

Table 1: Microbial load on the fish samples

Pathogens	Sample			
	Sardinella Longiceps **(Sample A)**	***Rastrelliger Kanagurta*** **(Sample B)**	***Stolepherus commersonni*** **(Sample C)**	***Leiognathus*** **sp. (Sample D)**
Total Heterotrophic Bacteria	4.8×10^4	3.9×10^4	4.1×10^4	5.2×10^4
Escherichia coli MPN/100g	27	21	33	26
Vibrio cholera MPN/100g	2	4	6	7
V.parahaemolyticus MPN/100g	8	9	12	17
Salmonella sp. MPN/100g	9	13	12	14
Shigella sp. MPN/100g	8	14	12	17

The maximum total heterotrophic bacterial count was observed in fish sample D (5.2×10^4) followed by sample A (4.8×10^4), sample C (4.1×10^4) and sample B (3.9×10^4) respectively. Whereas checking the *Escherichia coli* load on the maximum was observed in sample C (33) followed by sample A (27), sample D (26) and sample B (21) respectively (Fig. 5 & Table. 1).

Fungi

The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. In this study, different fungal strains such as, *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicillium* sp., *Verticilium* sp. and *Hemicola* sp. were isolated from all the four different fish samples (Fig. 6 & Table. 2).

Table 2: Presence of fungi in fish samples

Name of the Fungi	Sample			
	Sardinella longiceps	*Rastrelliger kanagurta*	*Stolepherus commersonni*	*Leiognathus* sp.
Aspergillus sp.	+	+	+	+
Mucor sp.	-	+	-	+
Fusarium sp.	-	-	+	+
Penicillium sp.	+	-	-	+
Verticilium sp.	+	-	-	-

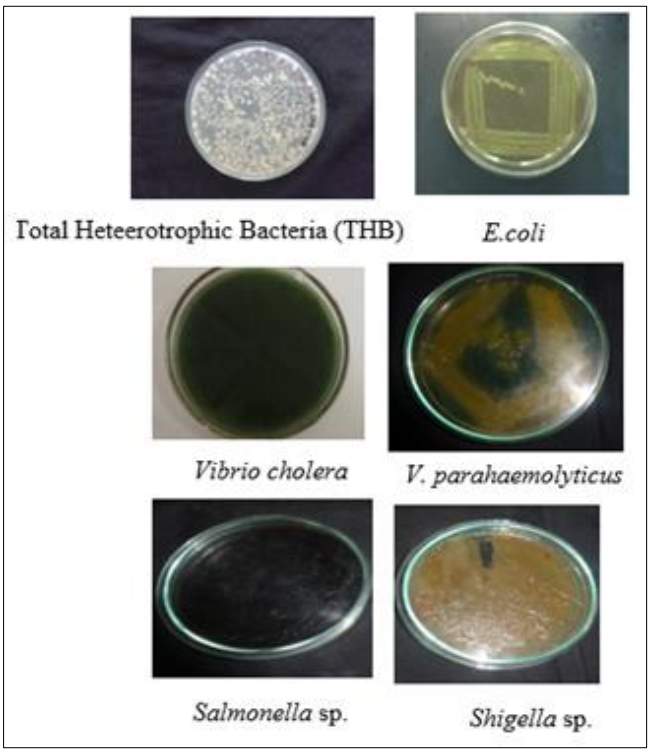

Fig 5: Isolation of bacterial pathogens from fish samples

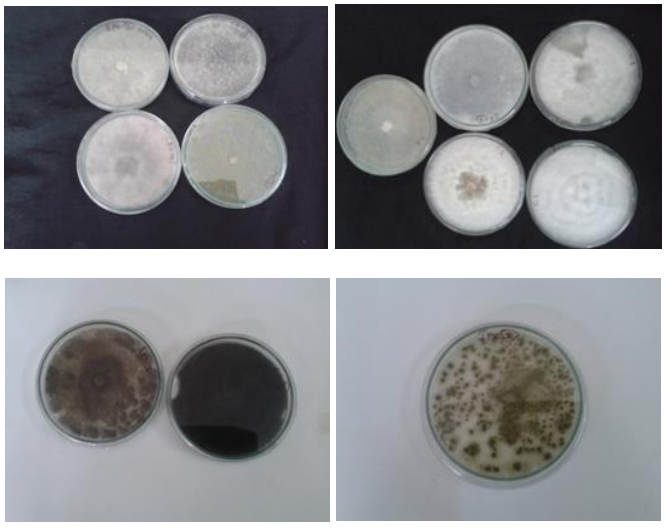

Fig 6: Fungal strains isolated from fish samples

Discussion

In the present study, the bacterial and fungal infestation were found from four different species like anchovy (*Stolephorous commersonni*), sardine (*Sardinella longiceps*), mackerel (*Rostrelliger kanagurta*) and silver bellies (*Leiognathus* sp.). The bacterial strains were total heterotrophic bacteria, *Escherichia coli*, *Vibrio cholera*, *V. parahaemolyticus*, *Salmonella* sp. and *Shigella* sp. The microbiology contamination was observed fresh naturally sun – dried samples, but the samples dried in solar dryer ware sterile due to low moisture content. Rillo *et al.*, (1998) [5] studied the microbial quality of commercially available dried mackerel of Philippines and reported presence of microbes. Microbial load in the samples from solar dryer was less due to clean and safe practice followed. In general, among the aquatic pathogens *Vibrio* species are highly dangerous and it will detached with shrimp epithelium and affect highly by eliminating the two layers which protects the shrimp from infections and finally end with high mortality (Martin *et al.*, 2004) [3]. The same like here the *Vibrio* sp. (*V. cholera* and *V. parahaemolyticus*) load also was found to be rich in all the samples and the maximum was found in sample (D). According to Reij *et al.*, (2004) [6] poor hygiene and unsanitary handling of food are the causes of microbial contamination. Leung *et al.*, (1992) [2] found that the catfish had a high viscera bacterial count and this was reflected in the microbial counts in the catfish flesh. Similarly, the *Salmonella* sp. and *Shigella* sp. load also have found higher in *Leiognathus* sp. compared to other three samples. Yusuf Ali *et al.*, (2012) [8] have studied and reported the total coliforms observed in Cooked IQF shrimp was <3 ±0.00 MPN/g, while it was 23.50 ± 13.72 MPN/g in raw block frozen shrimp. Fecal coliforms for both raw block frozen and cooked IQF shrimp were <3 MPN/g. The total plate count and *E. coli* counts were found to be higher in commercially sun dried fish (commercially sun dried fish) which is available in Tuticorin local market than the experimentally sun dried fish. High counts of TPC and *E. coli* in commercial sample was due to high content of moisture and humidity in the environment and unhygienic method of preparation. *Salmonella* was not detected in the raw and sun dried fish samples. Comparison of the total bacterial count of the fresh and dried fish samples showed that fresh *Oreochromis niloticus* had the highest colony count of 1.8x $10^7$and dried *Clarias gariepinus* had the lowest total colony count of 2.0 x 10^4which indicates that drying reduced the microbial load of the samples. In the present study the fungal strains were isolated from four different species of dried fishes. The fungal strains were *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicillium* sp. and *Verticilium* sp. The fungal species such as *Aspergillus* sp., *Mucor sp.*, *Rhizopus* sp. and *Fusarium* sp. are pathogenic to human beings (Felicia and Patterson, 2003) [1]. Sharma (1989) reported that *Aspergillus* sp., *Mucor* sp. and *Penicilium* sp. are known to cause food spoilage. Therefore, it is important to train local fisher folk on proper processing and preservation to improve the quality of the sun dried sea foods for the benefits of the consumers. Among that, fungal contamination is a common problem and it adversely affects the quality of cured fishes. Fish and fishery products are in the forefront of food safety because of their

indispensable role as cheap protein supplement and their significance as one of the most internationally traded foodstuffs. In recent times the number of food borne disease outbreak are rising (WHO, 2002) [7]. The total fungal count was also high in commercially sun dried fish sample (sample C) due to high moisture content and humidity. The cured fish in hot humidity tropical mould growth. Visible colonies appeared on the fish samples due to high moisture content and high relative humidity and atmosphere. The biochemical and microbial analysis showed that the quality of experimentally sun dried fish sample was good than the commercially sun dried fish sample. Experimentally sun dried fish was properly handled and well exposed to sun light and moving air and it dried quickly and the end product was clean and hygienic. The quality of processed dried fishes were found with microbiological infestation of different contaminants. Those obtained from retail markets were comparatively contaminated due to long storage, improper storage mechanism, etc. Caution should therefore be taken in consuming sun dried fish which have been displayed openly in markets because such fish could contain pathogenic microorganisms. Such products should be properly cooked before consumption. Continuous education of fish traders to use general good management practices and regular hygiene inspections by the standards authority is therefore required to improve the microbial quality of processed fish. Similarly, consumers should be continuously sensitized to raise awareness of the existence of such microbes in order to encourage adequate cooking of fish prior to consumption.

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