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Research Article **Zoology**

ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINATION IN INFECTED FISH AS *Sardina pilchardus*

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ABSTRACT

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 and noesis development and it also heavily affects the healthcare systems. In the present study was to investigate microbial contamination in infected *Sardina pilchardus* fish. By this study microbial analysis focusing on bacteria of the infected fish collected from Thanjavur market was performed. On the basis of morphological and analyzing various biochemical test results the genus of isolated strains were predicted as *Klebsiella neumonia, Escherichia coli, Staphylococcus aureus, Vibrio chlorae* and *Salmonella sp.* It was evident that produce from market showed highest level of contamination on all the parameters, thus it is essential to monitor that good hygienic practices should be incise at the market. The possible sources of these contaminants are due to the unhygienic manner of handling meat in the abattoirs. This implies that these meat and fish products are viable sources of various diseases. Some of these diseases could spread and acquire epidemic status which could pose serious health hazards.

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INTRODUCTION

Fishes are classified as any of the cold blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, 'fish' also refers to the flesh of such animals used as food. There are about 22,000 species of fish that began evolving around 480 million years ago (Pal and Mahendra, 2015). Fish is an important part of a healthy diet due to its high quality protein, other essential nutrients and omega 3fatty acids, and its low fat content as compared to other meats (Rhea, 2009; Pal, 2010).Fish and seafood products constitute an important food commodity in the international trade due to its ever increasing consumption demand. Fish contributes about 60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish (Emikpe *et al*., 2011). Fish allows for protein improved nutrition in that 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).

Live fish is normally considered to be sterile, but microorganisms are found in varying numbers on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish. A normal range of 102107 cfu (colony forming units)/cm2 on the skin and between 103and 109 cfu/g in the gills and intestines has been observed (Adedeji and Adetunji, 2004). When the aquatic system is contaminated with pathogenic Vibrio, these bacteria become part of the shellfish microflora (Colakoglu *et al.,* 2006), and when consumed with the fish the hazardous pathogenic Vibrio causes life threatening food borne infections and poses a considerable public health threat. Pathogenic Vibriobacteria are represented as important microbial agents ofsporadic and epidemic human infections in the field of food safety (Espineira *et al.*, 2010).

Most of the organisms found in fishes are associated with food poisoning infections in humans such as typhoid fever and shigellosis, whereas the presence of Aspergillus spp. reveals possible production ofaflatoxins (Pal *et al.,* 2015). High microbial load of wild catfish and wild tilapia was observed due to pollution of the environment in which the fish were caught (Emikpe *et al.*,2011). The presence of highly pathogenic bacteria such as Bacillus sp., Salmonella spp., Shigella spp., E. coli, Pseudomonas spp. and S. aureus is of public health concern, and indicates possible contamination resulting from the use of well water (Pal,2010). In the present study was to investigate microbial contamination in infected *Sardina pilchardus* fish.

MATERIALS AND METHODS

Collection of experimental fishes

Sardina pilchardus fish was procured from Fish market, Kelavasal, Thanjavur Tamil Nadu, India

Fig 1: Infected fish (*Sardina pilchardus*)

Nutrient broth (NAHimedia) Media for Bacteria

Preparation of bacterial broth culture:

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the broth completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well and pour into sterile conical flask and inoculate the sample. The inoculated broth was incubated at 37ºC for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 28˚C until use.

Fig 2: Bacterial broth culture

Broth **Culture**

Isolation of bacteria

The sample was pounded in a steriled porcelain mortar. 1gram of each sample was aseptically introduced into 9ml of peptone water in a universal bottle, to give 10^{-1} dilution and three subsequent serial dilutions were prepared in test tubes by transferring 1ml to 9ml of peptone water (Adegoke, 2004) and spreaded over the nutrient agar medium and the plates were incubated at 37°C for 24 hrs.

Microscopical examination

The morphological analysis of Microorganism was examined by using a sterile loop to pick culture from the culture plate were placed on a microscope slide, covered with a cover slip and observed under the microscope for structure.

Motility test using hanging drop slide

The motility test was performed to differentiate motile bacteria from non-motile one. Before performing the test, a pure culture of the organism was allowed to grow in Nutrient Broth (NB). One drop of cultured broth was placed on the clean cover-slip and was placed invertedly over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover-slip and to prevent evaporation of the fluid by air current. The hanging drop slide was then examined carefully under high power objective (100X) of a compound light microscope. The motile and non-motile organisms were identified by observing motility in contrasting with swinging movement of bacteria .

Bacterial colony counting

100 µl of diluted suspension was poured into the surface of Nutrient agar plate and spread by

„L" shaped spreader. The bacteria can thus be isolated and counted by C.F.U i.e. Colony Forming Unit. C.F.U=No. of colonies/inoculum size (g) X Dilution Factor

Biochemical characterization

The biochemical tests were conducted by the following methods, as described by Cappuccino and Sherman, (1999) to identify the bacteria.

RESULTS AND DISCUSSION

Bacteria, which are prokaryotic microorganisms, are the most abundant and simplest organisms in the world as we know it. Prokaryotes do not possess a nucleus and complex organelles. Because most prokaryotes range in size less than ten micrometers (µm), microscopes are used to study bacteria. Bacteria identification is very important in microbiology and pathology as it serves a basis of understanding diseases. Due to this, various types of methods have been introduced to classify bacteria in microbiology. Clinicians and microbiologists commonly employ the typing schemes which are dependent on the phenotypic typing schemes to develop the bacterial morphology and staining properties of the organism (Ying, et al., 2011).

The most basic technique used for classifying bacteria is based on the bacterium's shape and cell arrangement. The most ordinary shapes of bacteria include rod, cocci (round), and spiral forms. Cellular arrangements occur singularly, in series, and in groups. Some species have one to numerous projections called flagella which enable the bacteria to swim and move. Cocci or coccus for a single cell are round cells, occasionally flattened when being adjacent to each other. Cocci bacteria can exist individually, in pairs, in groups of four, in chains, in clusters or in cubes consisting of eight cells. Bacilli are rod-shaped bacteria which also can occur individually, in pairs, or in chains ((Prabakar et al., 2010).

Bacteria classification plays important role in yielding information for disease control. Bacterial species are usually sub-grouped to different types and is used for many crucial pathogenic bacteria such as *Salmonellae*, *E Coli*, and *Vibriones* (Frank, 2009). H.C. Gram in 1884 discovered the Gram stain classification remains an important and useful technique until today. This technique classifies bacteria as either Gram positive or negative based on their morphology and differential staining properties (Prabakar et al., 2010)..

Colonies

A viable cell is defined as a cell which is able to divide and form a population (or colony). A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions onto an appropriate culture medium, then incubating the plates under proper conditions so that colonies are formed. After incubation, the colonies are counted and, from a knowledge of the dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers.

Two hundred and twenty three (223) colonies were isolated, with different aspects: circular viscosity, sharp pointed and brilliant, opaque, pumpkin, white and cream colored. All the colonies were confirmed as bacteria of the non-fermentative gram-negative and positive group, strictly aerobic, positive for indole and negative for oxidase.

Total Bacterial Count

The isolated bacteria was quantified by calculating Colony Forming Unit (C.F.U) i.e. Colony Forming Unit. The obtained C.F.U values are represented in the following table . The numbers of bacterial colonies were isolated by pour plate technique. The highest bacterial populations were present in nutrient agar plate In the present study total bacterial density was range from 223 (CFU/g).

Fig 3: Bacterial colony

Table 1: bacterial colony counting in infected fish

Dilution	No. of Colonies	Dilution factor	CFU(per g)
10^4	223	10^4	$223x10^4$

Morphology characterization of bacteria

Five isolates were characterized on the basis of colony morphology and the staining characteristics. It was observed that four isolates were gram (-ve) rods and Bacilli and one isolate was gram (+ve) Cocci (Fig 2). Among the five isolates, two were motility rest of them are non motility.

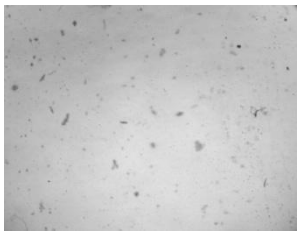
Table 3: Morphological characters of isolated bacterial species

Microorganisms	Morphology	Gram straining	Motility
Escherichia coli	Rod	-	M
Klebsiella pneumoniae	Bacilli	-	NM
Staphylococcus aureus	Cocci	+	NM
Vibrio chlorae	Rod	-	M
Salmonella sp.	Rod	-	NM

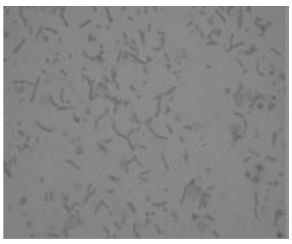
(+) Positive (-) Negative M= Motile NM= Non motile

Fig 4: Morphological characters of isolated bacterial species

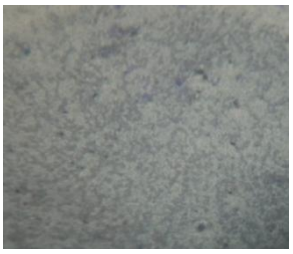
Escherichia coli



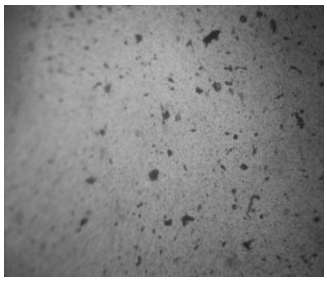
Vibrio chlorae



Klebsiella pneumoniae



Staphylococcus aureus



Salmonella sp.

Biochemical Characterization

Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. The five isolates were characterized on the basis of biochemical tests (Table 3). The tests performed to characterize the isolates were Indole, MR, VP and citrate test .

Table 1 Biochemical characters of bacteria

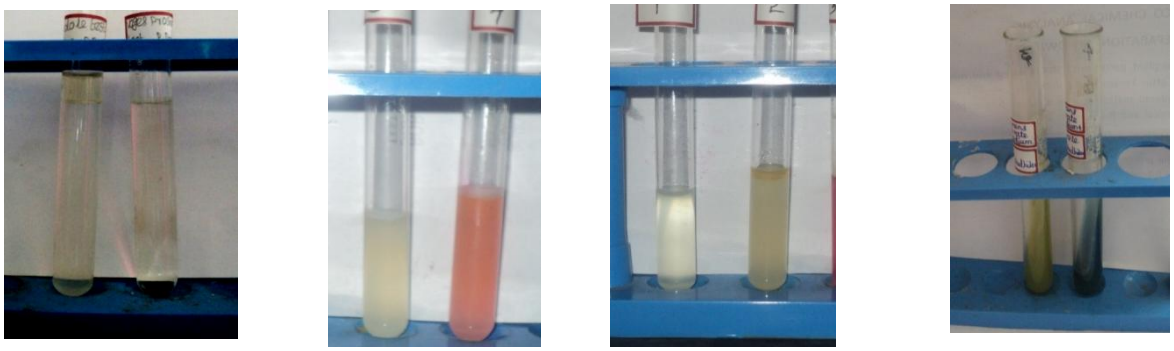
Microorganisms	Indole test	Methyl Red test	Vogesproskauer test	Citrate test
Klebsiella pneumoniae	-	+	-	+
Escherichia coli	+	-	-	-
Staphylococcus aureus	-	-	-	-
Vibrio chlorae	+	-	-	+
Salmonella sp.	-	+	-	-

(+) Positive (-) Negative

On the basis of morphology and biochemical characterization, the *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Vibrio chlorae and Salmonella sp.* were found to be in infected fish.

Fig 5 Biochemical characters of bacteria

Indole test **Methyl red test** **Vogesproskauer test** **Citrate test**



Contaminated raw meat and fish is one of the main sources of food-borne illness. It has been known that most food contaminations are caused by food-borne pathogens such as bacteria, fungi, mold and others. Keeping this in view, isolate and identify the type of bacteria present in fish products sold in Thanjavur market. By this study microbial analysis focusing on bacteria of the infected fish collected from Thanjavur market was performed. On the basis of morphological and analyzing various biochemical test results the genus of isolated strains were predicted as *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Vibrio chlorae* and *Salmonella sp.*

It was also evident that produce from market showed highest level of contamination on all the parameters, thus it is essential to monitor that good hygienic practices should be incise at the market. The possible sources of these contaminants are due to the unhygienic manner of handling meat in the abattoirs. This implies that these meat and fish products are viable sources of various diseases. Some of these diseases could spread and acquire epidemic status which could pose serious health hazards.

REFERENCES

Adedeji, O. B. and Adetunji, V.O. 2004. Pests in farm animals and stored animal products. agriculture, renewable natural resources, animal husbandry and health. Published by General Studies Programme (GSP), University of Ibadan. Nigeria. Pp. 141 – 151.

Adegoke G.O, Understanding Food Microbiology, 2nd Ed 2004 p.173-174.

Cappuccino, G.G. and Sherman, N., 1999. Microbiology a Laboratory Manual Rockland Aommunity College, Suffern: New York.

Colakoglu, F. A., Sarmasik, A. and Koseoglu, B.2006. Occurrence of Vibrio spp. and Aeromonas spp. in shellfish harvested off Dardanelles cost of Turkey. Food control 17: 648652.

Emikpe, B.O., Adebisi, T. and Adedeji, O.B. 2011. Bacteria load on the skin and stomach of Clarias Gariepinus and Oreochromis Niloticus from Ibadan, South West Nigeria: Public health implications. Journal of Microbiology and Biotechnology Research 1: 5259.

Espineira, M., Atanassova, M., Vieites, J. M. and Santaclara, F. J. 2010. Validation of a method for the detection of five species, serogroups, biotypes and virulence factors of Vibrio by multiplex PCR in fish and seafood. Food Microbiology 27: 122131.

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Frank L., "Bacterial Classification, Structure and Function", 2009, pp.1-2.

Pal, M. 2010. Fish hygiene. MSc Lecture Notes. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. Pp.111.

Pal, M., Girzaw, F., Abera, F., Shukla, P.K. and Hazarika, R. A. 2015. Mycotoxicosis: A growing concern to human and animal health. Beverage and Food World 42: 4246,50.

Prabakar S., et al., "Development of Image Processing Scheme for Bacterial Classification Based on Optima Discriminant Feature",Proceedings of the 2010 International Conference of Bioinformatics

Rhea, F. 2009. Microbiology handbook: Fish and seafood. Leatherhead Food International Ltd. Surrey, UK.

Ying Z, et al., "Bacteria Classification Using Neural Network", Institute of Biology Gansu Academy of Sciences, 2011