International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 9 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.09.004

Microbial pathogens in canned fish collected from Tamil Nadu

Palaniappan Dhinesh, Sekar Harikrishnan, Alager Kartick, Murugan Parivallal, Thirugnanasambandam Ramanathan, Singaram Jayalakshmi*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India.
*Corresponding author: *jayacas@gmail.com*

Abstract

The present investigation was on testing for the pathogenic microbial contamination and quality of canned fish products purchased from departmental stores of various places in Tamil Nadu. Three different canned fish products namely A, B and C were examined for microbial contamination using different selective media. Results of the present study showed that none of the canned fish sample tested was sterile and it showed the presence of different pathogenic bacteria such as *E. coli, Staphylococcus aureus, Salmonella spp, Vibrio spp, Listeria spp.* The total viable count (TVC) of *Vibrio spp*. in canned product A, B and C were 1.2 x 10^2 to 2.5 x 10^3, 1.8 x 10^2 to 2.3x10^3 and 2.0x10^2 to 2.8x 10^3 CFU/g respectively. Likewise, *S. aureus* in product A, B and C were 1.4 x 10^1 to 2.7 x 10^2, 1.0 x 10^1 to 2.0 x 10^2 and 1.3 x 10^2 to 3.0 x10^2 CFU/g respectively. *Salmonella* spp. in product A, B and C were respectively 1.1 x 10^2 to 2.3 x 10^2, 1 to 1.0x10^2 and 1.0x 10^1 to 2.8x10^2 CFU/g. *E. coli* in product A, B and C was respectively 1.3 x 10^2 to 2.5 x 10^3, 1 to 1.0x10^3 and 1.8x $10^2$to 3.0x10^3 CFU/g. *Listeria* spp. was in the range of 0.9 x 10^1 to 1.5 x 10^2, 1.0x10^1 to 1.0x10^2 and 1 to 1.8x10^3 CFU/g in product A, B and C respectively. This study has shown the presence of microbial contamination in the tested canned fish product.

Keywords: Canned fish, *E. coli, Staphylococcus aureus, Salmonella* spp., *Vibrio* spp., *Listeria* spp. Total viable count.

Introduction

Seafood is well known for its nutritive values, minerals, and vitamin contents such as vitamin A, B, D, and omega-3-fatty acid. Generally, the fisheries products are marketed in fresh forms, fresh fishes are fishes which does not undergo any form of preservation treatment except the cooling process (BSN *et al.*, 2013). However, Fish and fishery products are not only known for their nutritive value, but they also play a vital role in the global trade as a foreign exchange between many countries in the world (Yago *et al.*, 2003). Approximately 10-12 percent (870 millions) of the people depends on the fisheries and aquaculture sectors in the world. Fishes have been commercially and successfully canned for more than hundreds of years. Canning is one of the most popular methods in the world for the preservation of fish and the shelf life ranges from 1 to 5 years. Canned Fish are sealed in airtight containers such as tins (Ania *et al.*, 2019). Canned products are most commonly consumed by the people who travel on ships. Furthermore, the majority of canned fishing items are made using vegetable oils and seawater. Preservation methods like as canning and freezing are employed due to the expense and lack of availability of

equipment and cold storage systems (Eya *et al.*, 1998). Most commonly this food can become contaminated during the process of storing. Pathogens such as *Vibrio* spp and *Salmonella* spp, naturally found in any aquatic environment can lead to contamination (Gnan *et al.*, 2005). Using such contaminated items may lead the customer who consumes them to get infected. This study was thus carried out to evaluate the microbiological safety of canned marine goods in order to increase the concern about food safety.

Materials and Methods

Chemicals and media used

All the chemicals used in the present study were purchased from the Himedia laboratories, India.

Sample collection

Different canned fish products were purchased from departmental stores from varies places in Tamil Nadu and shifted to laboratory in CAS in Marine Biology Portonovo for further analysis.

Sample processing

10g of sample from each can was transferred to 90ml of sterile distilled water and designated as 10^{-1} dilution. Further serial dilutions was done up to 10^{-4} using sterile distilled water.

Microbiological analysis of the canned seafood samples

Isolation of pathogens like *E. coli, Staphylococcus aureus, Salmonella* and *Shigella spp, Vibrio , Listeria* was done using Esoin Methylene Blue (EMB) agar, Manitol Salt Agar (MSA), Salmonella Shigella agar, Thiosulfate-Citrate-Bilesalt-Sucrose agar , PALCAM agar respectively adopting standard procedure. Spread plate technique was followed and plates were kept at 37°C incubation for 24 to 72 hours. Different pathogens present in the canned fish samples were calculated as the total viable count (TVC) and were expressed as CFU/g of sample.

Biochemical identification of bacterial strains

Biochemical tests are tests, which are used for the identification of different bacterial species, based on their biochemical activities (Thillai *et al.*, 2021)

Indole production test

It is used determine the capability of the bacteria to split the amino acid tryptophan into indole compounds.

Methyl red test

Methyl red (MR) test used to determine the Efficient production of acid during the process of fermentation of glucose. *MR positive*: The cultured media turns red in color after the addition of methyl red (pH at or below 4.4). *MR negative*: The cultured media will remain the same yellow color after the addition of methyl red. This is because of the higher pH value.

Voges-Proskauer test (VP)

This test is used to detect the presence of acetone in the culture broth. *VP positive*: If the culture media is positive, then the brownish-red color changes into pink in color. *VP negative*: If the culture media is negative, then there is no change in the color of the media.

Citrate utilization test

It is an ability of an organism, which uses the citrates as a sole carbon source and ammonium ions as the sole nitrogen source. *Citrate positive*: If it is positive then the growth will be seen in the test tube (slant surface) and the color changes to dark blue. *Citrate negative*: There is no growth will be seen and there is no change in the color (deep green color).

Triple sugar iron (TSI)

It is used to differentiate the enteric gram-negative bacteria, including *Salmonella*. *Alkaline*: The medium will be red in color before the reaction. *Acidic*: The medium will be yellow in color after the reaction.

Confirmative tests

Peptone water surface pellicle test (*Vibrio spp.)*

In peptone water, development begins as a fine surface pellicle that splits up into membranous fragments after shaking

Glucose fermentation (*Salmonella spp.*)

A layer of mineral oil is added to the top of the deep in one of the tubes to create anaerobic conditions. Oil is not added to the other tube to allow for aerobic conditions. The tubes are then incubated for 24–48 hours. If the medium in the anaerobic tube turns yellow, then the bacteria was confirmed as salmonella.

Microplate technique of hemolytic activity (*Listeria spp.*)

Hemolysis produced by *Listeria spp.* on blood agar is frequently difficult to interpret, we developed a microplate technique for the routine determination of hemolytic activity with erythrocyte suspensions.

Gas production test (*E. coli*)

The traditional method for the confirmation of *Escherichia coli* in routine coliform analysis in water laboratories has been to test for gas production at an elevated incubation temperature, either 44 or 44·5°C in this incubate period bacteria was producing gas.

Tube coagulase test (*S. aureus*)

Tube coagulase test detects free coagulase (staphylocoagulase) which reacts with coagulase-reacting factor (CRF). *CRF is a thrombin-like molecule*. Staphylocoagulase and CRF combine to indirectly convert fibrinogen to fibrin. A suspension of the organism is suspended and incubated with plasma at 37°C. Clot formation within 4 hours indicate its confirmed.

Results and Discussion

Since the canned samples, were already processed only limited samples were considered for the study. A total of 10 canned fish fresh sample collected at three different places (A-3, B-3, C-4) were taken for analysis and the density was calculated on average according to the given sample number. The present study results showed that none of the canned fish sample tested was sterile and it showed the presence of different pathogenic bacteria such as *E. coli*, *Staphylococcus aureus*, *Salmonella spp*, *Vibrio spp*, *Listeria spp.* The total viable count (TVC) of *Vibrio spp*. in canned product A, B and C were 1.2 x 10^2 to 2.5 x 10^3, 1.8 x 10^2 to 2.3x10^3 and 2.0x10^2 to 2.8x 10^3 CFU/g respectively. Likewise, *S. aureus* in product A, B and C were 1.4 x 10^1 to 2.7 x 10^2, 1.0 x 10^1 to 2.0 x 10^2 and 1.3 x 10^2 to 3.0 x10^2 CFU/g respectively. *Salmonella* spp. in product A, B and C were respectively 1.1 x 10^2 to 2.3 x 10^2, 1 to 1.0x10^2 and 1.0x 10^1 to 2.8x10^2 CFU/g. *E. coli* in product A, B and C was respectively 1.3 x 10^2 to 2.5 x 10^3, 1 to 1.0x10^3 and 1.8x $10^2$to 3.0x10^3 CFU/g. *Listeria* spp. was in the range of 0.9 x 10^1 to 1.5 x 10^2, 1.0x10^1 to 1.0x10^2 and 1 to 1.8x10^3 CFU/g in product A, B and C respectively.

All the above Sea food borne microbes were isolated in different canned products collected in various places in Tamilnadu. Nowadays in fishery industries, canning of fishery products is one of the most important factor. In the year of 1938, the total market value of all fish and fishery products was estimated about $214000000. In this, the fish caning industry accounted for 39% ($8344600) of this total value. Because to a variety of characteristics, including the nature of the habitat from which they come, their style of feeding, the season during which they are collected, and how they are cooked and served, some seafood commodities are intrinsically riskier than others. Pathogens can be acquired by fish, mollusks, and crustaceans from a variety of sources.(Sara et al., 2018). In the United States, 160 species of fishes are consumed regularly for food in that 160 species, only 15 species are canned in large scale. Although the canning industries were developed more in the industrialized countries of Northern hemisphere and also in some tropical countries produce different varieties of canned fish products. Taking an example, in Mexico and Brazil canned fishes are produced in large quantities and it is sold on large proportion in the local markets. In the world Morocco is reported as the largest producer of canned Sardines and Thailand is also now a producer of canned tuna fish in large quantities (Nahian *et al.*, 2017).

Table 1: Total viable count (TVC) of bacterial pathogens in canned fish products (in CFU/g)

Sample	Pathogens	Minimum	Maximum
Canned product A	*Staphylococcus aureus*	1.4×10^1	2.7×10^2
	Vibrio spp.	**1.2×10^2**	**2.5×10^3**
	Salmonella and *Shigella* spp.	1.1×10^2	2.3×10^2
	E. coli	1.3×10^2	2.5×10^3
	Listeria spp.	0.9×10^1	1.5×10^2
Canned product B	*Vibrio* spp.	1.8×10^2	2.3×10^3
	Staphylococcus aureus	1.0×10^1	2.0×10^2
	Salmonella and *Shigella* spp.	0(1)	1.0×10^2
	E. coli	0(1)	1.0×10^3
	Listeria spp.	1.0×10^1	1.0×10^2
Canned product C	*Vibrio* spp.	2.0×10^2	2.8×10^3
	Staphylococcus aureus	1.3×10^2	3.0×10^2
	Salmonella and *Shigella* spp.	1.0×10^1	2.8×10^2
	E. coli	1.8×10^2	3.0×10^3
	Listeria spp.	0(1)	1.8×10^3

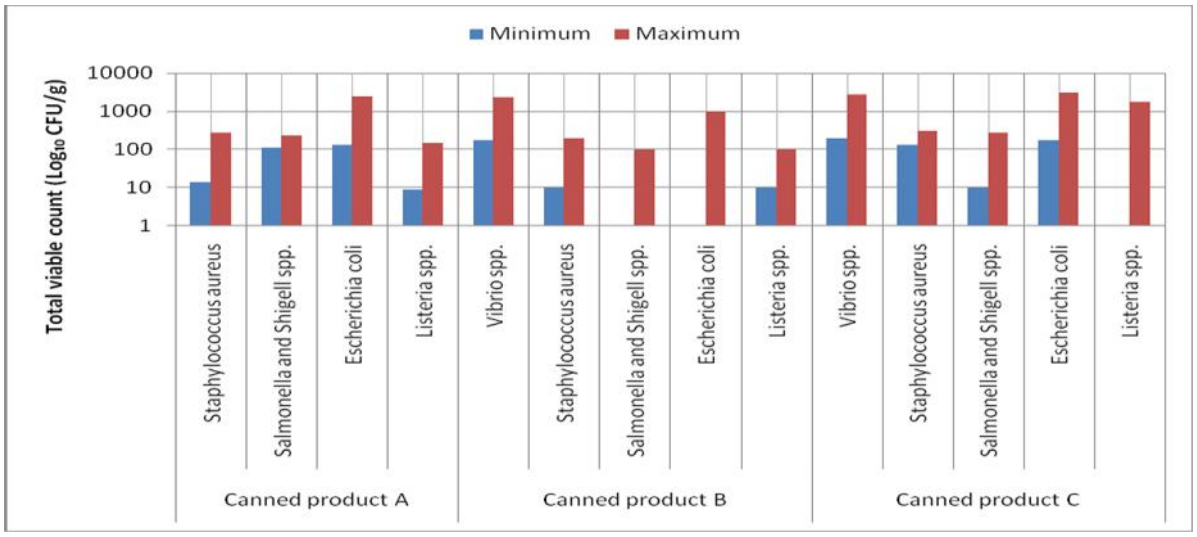

Fig. 1: Total viable count (TVC) ranges (minimum and maximum) of bacterial pathogens in canned fish products (in CFU/g)

On the **Figure 1**, canned fish fresh sample collected at three different places. On TCBS Canned Product A [03] was the first to have many bacteria colonies than other places followed by Canned Product C [09] whereas Canned Product B was the last where only [07] colonies growth was observed. On SSA medium and MSA sample collected at Canned Product A is the first to have a large number of bacteria colonies, Canned Product C is the second [08,07] followed by Canned Product B [13,10] and lastly was canned product A with [11,13] bacteria colonies growth. On EMB culture medium and PALCAM medium also the bacterial colonies growth was abundant in the sample collected from Canned Product A [10,13], followed by Canned Product C [11,07], the last one was Canned Product B with [16,10] bacterial colonies

Table 2: Biochemical identification of bacterial pathogens from canned fish products

Indole Test	Methyl Red Test	Voges-Proskauer Test	Citrate Test	Gram's staining	H2S	Bacteria name
Negative	Positive	Negative	Positive	Negative	Negative	*Vibrio* spp.
Negative	Positive	Positive	Negative	Positive	Negative	*Staphylococcus aureus*
Negative	Positive	Negative	Negative	Positive	Positive	*Salmonella* spp.
Positive	Positive	Negative	Negative	Negative	Negative	*Escherichia coli*
Negative	Positive	Positive	Negative	Positive	Positive	*Listeria* spp.

Table 3: Biochemical identification of bacterial pathogens from canned fish products (Confirmatory tests)

Bacteria Name	Test Name	Result
Vibrio spp.	Peptone water surface pellicle test	Surface pellicle was present
Staphylococcus aureus	Glucose fermentation	Turns yellow (Positive)
Salmonella spp.	Hemolytic activity	A and β hemolysis
Escherichia coli	Gas production test	Gas production present
Listeria spp.	Tube coagulase test	Staphylo coagulase

Conclusion

The present study showed that none of the canned sample was sterile. Tested samples showed the presence of different pathogenic bacteria such as *E. coli, Staphylococcus aureus, Salmonella spp, Vibrio spp, Listeria spp.* In samples where one pathogen is low in density, others also found to be low. This was true in the samples of higher density also. As samples belonged to different brands, these differences might be due to the production process itself. Though, proper canning process destroy most of the microbes present in the fish samples used for the processing, inadequate processing including heating, cooling and improper sealing etc. even the quality changes happened in fish, before processing also influence the quality of canned fish. Although, all samples analysed were taken from perfect cans, without any blown or leaky conditions and well before the expiry date, the initial quality and the processing methods might have influenced the quality of the canned fish product.

Acknowledgments

The author gratefully acknowledges Annamalai University, Tamil Nadu, India for providing lab facilities and supporting our research.

Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare no conflict of interest.

References

Aarthi. G, Harikrishnan.S, Sudarshan.S, Karthick. A, Parivallal. M., Jayalakshmi. S Optimization of culture conditions for phenol degrading fungi *Penicillium notatum* SJ-04 isolated from industrial polluted East coastal area of Tamil Nadu., Journal of Interdisciplinary Cycle Research ISSN NO: 0022-1945Volume XIII, Issue VI, June/2021

BSN (Badan Standardisasi Nasional) 2013, Standar Nasional Indonesi (SNI) 01-2729-1-2013 tentaing spesifikasi Ikan Segar .Badan Standardisasi Nasioal Jakar

Pierre, BJ , and Sivasubramani ,K (2015) Assessment of microbiological safety of street orange fresh fruit juice sold at Chidambaram, India Int. J. Adv. Res. Biol. Sci. 2(10): (2015): 711

Parivallal, M., Harikrishnan, S., Kartick, A., Jayalakshmi, S., 2020 antibacterial activities of striped snakehead murrel fish *Channa striata* autochthonous gut bacterium achromobacter xylosixidans against bacterial fish pathogens., IJSR ,1159 -1164

Yagoub, S.O. and T.M Ahmed ,"Pathogenic microorganisms in fresh water samples collected from khartoum central market", Sundan Journal of Veterinary science and Animal Husbandry,vol 43,no.1 -2,pp 32-37,2003.

Thillainayagi,S., Harikrishnan,S., & Jayalakshmi, S., Screening optimization and production of uricase from *Alcaligenes faecalis* isolated from poultry farm litter (IJARESM), ISSN: 2455-6211 Volume 9, Issue 4, April -2021.

The ofania N.Tsironi, Petros S. Taoukis,in reference module in Food science,2019.

Eyabi GD (1998) Techniques for Fish Handling, Marketing and smoking in Cameroon.FAO Fisheries Reports 574:198-206.

Gnanambal.K and Patterson.J "Biochemical and microbiological quality of frozen fishes available in Tuticorin supermarkets".Fishery Technology,Vol 42 ,no.1 ,pp 83 - 84 ,2005.

Les bratt: GLOBEFISH consultant TECHNICAL GUIDE TO FISH CANNING GLOBEFISH research programmer,vol 111.rome,FAQ 2013.69p

Hutson, Anne (2008). "Oxidative-Fermentative Test Protocol". *American Society for Microbiology*.

Maarit Niemi,R Mentu, A. Siitonen, S.I. Niemelä Confirmation of Escherichia coli and its distinction from Klebsiella species by gas and indole formation at 44 and 44.5 degrees CJ Appl Microbiol. 2003;95(6):1242-9. doi: 10.1046/j.1365-2672.2003.02125.x.

Dominguez Rodriguez L, Vazquez Boland J A, Fernandez Garayzabal J F, Echalecu Tranchant P, Gomez-Lucia E, Rodriguez Ferri E F, Suarez Fernandez G, Microplate technique to determine hemolytic activity for routine typing of Listeria strains J Clin Microbiol 1986 Jul;24(1):99-103. doi: 10.1128/jcm.24.1.99-103.1986.

Clinical Microbiology Procedures Handbook, Fourth Edition. (2016). American Society of Microbiology. https://doi.org/10.1128/9781555818814.

Katz, D. S. (2010, November 11). *Coagulase Test Protocol*. https://www.asmscience.org /content/education/protocol/protocol.3220.

Sara.S, Saeid.S, Microbiology of fish and sea food,(2018) The first national congress on recent advance in engineering and modern sciences

Access this Article in Online

Website: www.ijarbs.com

Subject: Microbiology

Quick Response Code

DOI:10.22192/ijarbs.2021.08.09.004

How to cite this article:
Palaniappan Dhinesh, Sekar Harikrishnan, Alager Kartick, Murugan Parivallal, Thirugnanasambandam Ramanathan, Singaram Jayalakshmi. (2021 Microbial pathogens in canned fish collected from Tamil Nadu. Int. J. Adv. Res. Biol. Sci. 8(9): 23-29.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.09.004