International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 31-01-2022, Accepted: 15-02-2022, Published: 02-03-2022
Volume 7, Issue 1, 2022, Page No. 5-9

Microbial quality of different dried fishes collected from fish market of Parangipettai, Tamil Nadu

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Abstract
Dried fish is a popular diet, particularly among lower-income groups, because it is less expensive than other processed seafood. In the present study, the pathogenic bacterial contamination and quality of different dried fishes collected from different fish markets in Parangipettai, Tamil Nadu, were investigated. Ten different dried fishes are analyzed for bacterial or microbiological contamination using Zobell marine agar. The present study showed that the entire dried fish sample examined was contaminated. Identification of different pathogenic bacteria and percentage of bacterial occurrence was observed as *Staphylococcus* spp (8.9%), *E.Coli* (22.9%), *Bacillus* spp (12.7%), *Pseudomonas* spp (7.0%), *Salmonella* spp. (12.7%), *Lactobacillus* spp (5.7%), *Vibrio* spp (10.8%) unidentified bacteria isolated from dry fish (9.3%). The microbial density of different dried fishes *Mugil cephalus* 2.2×10^2, *Rastrelliger kanagurta* 1.7×10^2, *Sardinelle fimbriata* 1.0×10^3, *Nemipterus japonicas* 1.9×10^1, *Sepia osculenta* 3.1×10^2, *Macrobranchium rosenbergii* 4.0×10^1, *Dasyatis pastinaca* 2.0×10^3, *Carcharodon carcharias* 1.5×10^2, *Trichiurus lepturus* 2.8×10^2, *Litopenaeus vannamei* 3.3×10^1observed. This study concludes the pathogenic bacterial contamination in the ten different types of dried fish products.

Keywords: Dried fishes, *Staphylococcus spp, E.coli, Bacillus spp, Pseudomonas spp, Salmonella spp, Lactobacillus spp, Vibrio spp*

Introduction
One of the most important sources of animal protein for humankind is fish, which is globally accepted as a good protein source with other elements to maintain good health (Ravichandran *et al*., 2012) [2]. Dry fish occupied 25% of the total fish in the market (Islam *et al*, 2006) [13]. Salting and drying is an ancient method of preservation globally, also known as "Salt curing" (Sanjeev *et al*.,1996 and Anon*et al*.,2001) [18]. It is also a simple and oldest, and most economical method of fish preservation (Balachandran *et al*, 2001). Rural coastal areas are following this method, specifically using cheap fish. Moreover, it's a more affordable food for the poor throughout our country and other developing countries nearby (Chakrabarti *et al*, 1999 and Nesan *et al*.,2021) [5]. Dried fish quality depends on organoleptic, microbiological, and biochemical quality conditions (Azam *et al*.,2003). Being a highly perishable product, fish to be preserved immediately (Bala *et al*.,2001). In India, sun drying and salt drying are considered as the best option for low-valued fishes in 2013-2014, and dry fish export contributed up to 7.86% (819 cores) of all forms of fish exports (MPEDA, 2014). The dried fishes retain higher quality standards when compared to fresh fishes and the nutritional quality of dry fish remains unchain (Frauque MO.,2012).

Materials and Methods
Study areas and period
In the present study, the common bacterial strains were isolated and identified from dry fish samples, which were purchased from the fish market of Parangipettai. This research was conducted in CAS in marine biology, Annamalai University, from Aug-2021 to Oct-2021.

Collection of sample
Ten different dried fish samples were collected from different fish markets in Parangipettai, Cuddalore district. *Mugil cephalus, Rastrelliger kanagurta, Sardinella fimbriata, Nemipterus japonicus, Sepia osculenta, Macrobranchium rosenbergii, Dasyatis pastinaca, Carcharodon carcharias, Trichiurus lepturus and Litopenaeus vannamei* were collected in a separate sterile polythene bag and shifted to Research laboratory in CAS in Marine Biology Parangipettai for further analysis.

Processing of Samples

10g of minced dry fish sample was added to 90 ml of 50% aged seawater mixed well and designated as 10^{-2}. Further serial dilutions were done up to the level of 10^{-7}. Whenever the sample was found to be turbid, centrifugation was done at 3500 rpm for 5 min. The supernatant was used for further studies.

Isolation and Identification of bacteria from samples

The consecutive sequentially diluted three samples were plated on Zobell marine agar (ZMA), and after incubation at 28 ± 2ºC for 24-48 hrs, they were observed for morphology. Density was calculated, and pure cultures were done and identified according to Bergey's manual of determinative bacteriology (Buchanan *et al*., 1974) Hemolytic activity described in Gerhardt and al. (1994) and biochemical test viz sugar fermentation (Cheesbrough, 2006), Catalase testing, Indole testing, methyl red (MR) test, and Voges-Proskauer (VP) test, Simmon citrate, Cytochrome oxidase Motility (SIM), Gram's staining, carbohydrate fermentation, starch, lipid and protein hydrolysis were used to identify the bacteria. (Merchant and Packer *et al*., 1967; Dhinesh *et al*.,2021)

Maintenance of stock culture

The pure cultures obtained were streaked on the Zobell marine agar slants and used for further study. However, one set of agar slants was kept in a refrigerator for future studies.

Results and Discussion

Isolated bacteria and cultural characterization

In tropical areas, the temperature is high, which leads to quickly spoiled food, so it's more important to cool things down right away or salt them to avoid microbial spoilage (Jeya *et al*., 2003). Reduced water activity, microbial growth, and spoilage are very much limited for the dried fish (Daramola *et al*.,2007). Drying the fish in the sun is one of the oldest methods of fish preservation, and this process will take more time. Because of this long process, an alternative method of salt curing was adopted (Goswami *et al*.,2002). When the temperature decreases during the monsoon season, the drying process cannot be done by the traditional method. During this season, the fish absorb the moisture and act as habitats for microbial populations (Azam *et al*.,2002).

Dried fishes are the popular diet of people, especially of the lower-income group, as it is comparatively cheaper than other kinds of processed seafood. In the present investigation were estimated and 100% of the samples were contaminated with all the pathogens studied. Aerobic plate count was in the range of minimum 1.9×10^{1} in *Nemipterus japonicas*, among *Dasyatis pastinaca* 2.0×10^{3}. All the samples tested, results of all the APC were in the acceptable range of $10^{1,}$ whereas 10^{3} of them exceeded and observed to the level of the higher APC coupled with the presence of pathogens indicated the unhygienic practices involved in production. Though the facility is available to dry the fish in raised platforms, during the season where the catch is more sun-drying on sand could also be observed. It is common to wash the fish in coastal waters before giving a rinse in freshwater. Fishes like *Mugil cephalus* were bigger in size, and adequate drying was not there due to frequent rain during the collection period. Even well-dried forms have also been become wet again due to improper storage. The fisherfolk were not fully sensitized irrespective of training programs arranged from time to time by CAS in Marine Biology, Parangipettai about hygienic practices in the handling of fishes. More facilities to be created for solar drying in controlled conditions. The work done by savitha et results al., 2012, Ginigaddrage *et al*., 2018 respectively in India and Sri Lanka also showed similarly. Inadequate salting may also be a reason as the growth of *E. coli*, *Staphylococcus spp* were not inhibited. Even at 18% NaCl, *S.aureus* is capable of growing, and the work done by in 22% *E. coli* cells were damaged in a higher concentration of salt, which contradicts the present results. Quality of the salt is also an essential factor in the drying process. *Mugil cephalus* and *Rastrelliger kanagurta* are the common fishes used for sun-drying and salt curing on the Parangipettai coast. Irrespective of countries, whether high or low in economic status, low-valued fish are used for sun drying or salt curing. Still lower, or categorized as trash fish, is used for meal preparation. As the fish meal is also used for aquaculture and cattle rearing, those fish used for that production are also important. In this regard, the evaluation of sun-dried or salt-cured fish deserves appreciation. The present study, along with other studies worldwide, insists that the hygienic practices in seafood processing and the stable holders like the fisherman, fisherfolk, auctioneers, truck owners, driers, and the general public, especially the younger population, should be sensitized in this regard. Research institutes, especially in coastal areas, teaching fisheries, aquaculture, etc., should view this service to humankind by sensitizing the stakeholders, especially those who don't have or have less knowledge in these aspects. *E. coli* contamination of *Nemipterus japonicus*, *Dasyatis pastinaca*, and *Litopenaeus vannamei* was higher in 22.9 percent of the samples examined. The variation in quality might be due to the fact that they were purchased from different vendors whose practices may differ, as human handling also plays a significant role in determining the microbial quality of fresh as well as processed seafood products. Another reason may be dust with infected aerosols. The recent pandemic of COVID worldwide emphasizes the need for preparedness against deadly pathogens or even less pathogenic forms that may be treated if environmental issues become favorable to them, predisposing the host to fatal diseases. The presence of pathogens in all samples indicated the poor water quality of coastal waters in this area coupled with unhygienic practices. Though the study is limited in a few aspects seems to be an eye-opener regarding the seafood quality of this coastal area. To overcome this, practices like smoke drying can also be tried.

Morphological characterizations

Morphological characterization was done by in plates and using colony morphology Gram's staining technique on individual pure culture (Gram, 1884), and the results for different bacterial isolates are represented in Table 2 In the study the most common occurrences *Staphylococcus* spp (8.9%) were followed by E.*Coli* (22.9%), *Bacillus* spp (12.7%), *Pseudomonas* spp (7.0%), *Salmonella* spp. (12.7%), *Lactobacillus* spp (5.7%), *Vibrio* spp (10. 8%), and unidentified bacteria isolated from dry fish (12%). The same percentage of events (Sultana *et al*., 2010) recorded no report on *E. coli* for *Staphylococcus* spp., *Bacillus* spp., and *Salmonella* spp. in the research. *Listeria spp* greatest incidence in dried fish is the lowest minimum concentration among microorganisms discovered in this study (Lecocq *et al*., 2020). Also previously found in dried fish samples were *E. coli, Salmonella spp., Listeria spp.,* and *Staphylococcus spp.* (Hasan *et al*., 2016; Hussain *et al*., 2018 and Nur *et al*., 2020). Intentionally poor drying might lead to microbial development in dried fish since full drying causes weight loss. Dry fish is particularly polluted due to inadequate packing, hygiene, and sanitation procedures from harvesting to marketing level, although (Paul *et al*., 2018). In addition, the utilization of inferior raw fish for drying is also seen as a role in microbial development (Hasan *et al*., 2016). *Salmonella* spp. is clearly shown to be absent from sanitary procedures in this research (Sultana *et al*., 2010; Nur *et al*., 2020) *Listeria spp* and *Bacillus spp* is a source of marine environmental contamination in dry fish. Sundryers may also decrease but are not efficient in destroying the microbial burden of bacterial spores (Paul *et al*., 2018). Improper packing and storage systems may be recognized as one of the key microbial contamination reasons (Islam *et al*., 2020).

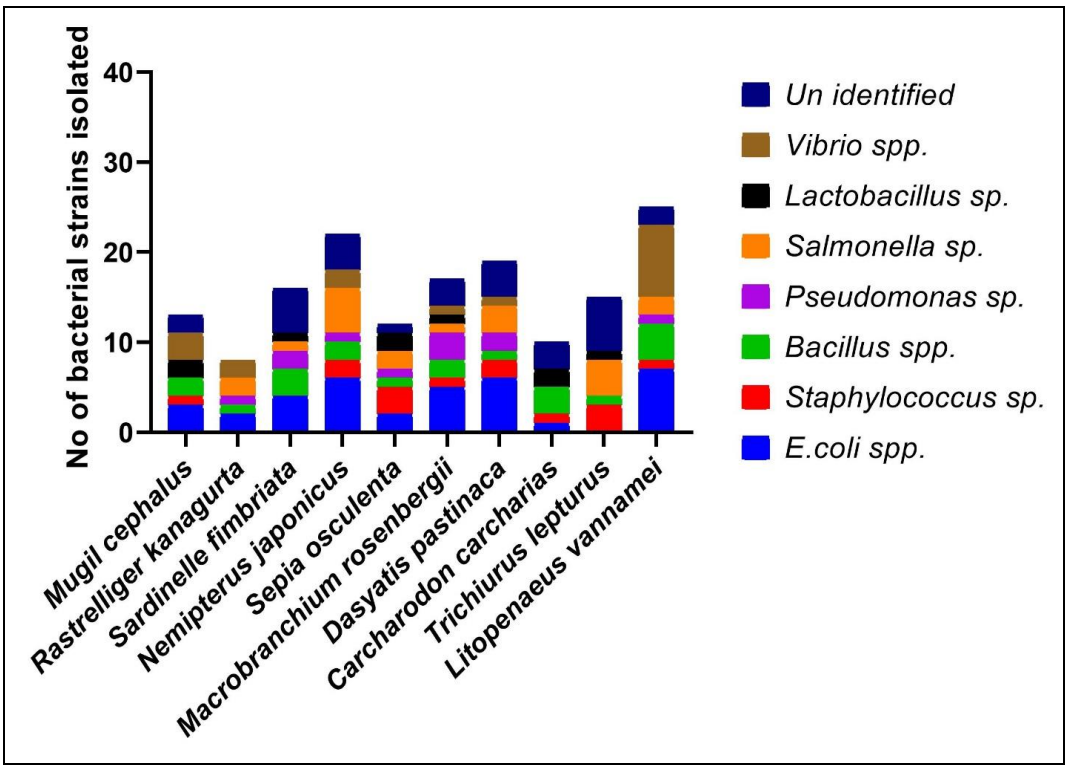

Fig 1: Prevalence of the isolated bacteria from different fish and shellfish sample

Table 1: Colony features of isolated bacteria on culture media

Bacteria	Media	Colony of characteristics
E.coli	Zobell Marine Agar	Large, Thick, Grayish white, Moist, Smooth, Opaque
	EMB medium	Large, Blackish blue with green metallic sheen
Bacillus spp.	Zobell Marine Agar	circular, Opaque, Rough with jagged edges, some colonies were large grey white and granular with wavy edge
	BA medium	Grey-white, Opaque colonies with Beta hemolysis
Staphylococcus sp	Zobell Marine Agar	Transparent,whitish, Yellowish, Orange color and Smooth colonies.
	BA medium	No hemolysis but pigmented colonies observed.
Salmonella spp.	Zobell Marine Agar	Opaque and smooth colonies with 2-4 mm in diameter.
	SS medium	Translucent, Smooth, Round with black center
Lactobacillus spp	Zobell Marine Agar	2-4 mm in diameter, white shiny appearance, Round shaped
	MRS medium	2-5 mm in diameter round, convex/domed with Entiremargin, Smooth and shiny surface, cream ring with tinted blue center due to acid production the bromophenol blue in the agar tubs green/yellow
Vibrio spp.	Zobell Marine Agar	Glistening and translucent colonies, 1-2mm in Diameter after 18-24 hrs smoothly and shiny

	TCBS Medium	Yellow and shiny colonies, 2-4 mm in diameter
	BA Medium	colorless colonies, with hemolysis
Pseudomonas spp	ZMA medium	Colonies were smooth, large, translucent and low convex 2-4 mm in diameter, Greenish Blue pigment diffused.
	BA Medium	Colonies are smooth, large, translucent, low convex, Hemolytic, greenish blue pigment diffused.
	Cetrimide Medium	Large, Flat, Spreading colonies, Hemolytic, Pigment Producing, Dark greenish blue color

Table 2: Biochemical characteristics of bacteria isolated from the different fish sample

S. No	Bacteria	Staining properties	Shape	Category	Motility	Catalase	Indole	MR	VP	Citrate	Oxidase
1	*E. coli spp.*	Pink	Rod	- Ve	Motile	+Ve	+Ve	+Ve	-Ve	- Ve	- Ve
2	*Staphylococcus spp.*	Violet	Coccus	+Ve	Non-Motile	+Ve	-Ve	+Ve	+Ve	- Ve	+Ve
3	*Bacillus spp.*	Violet	Large bacillus	- Ve	Non-Motile	+Ve	-Ve	+Ve	- Ve	+Ve	-Ve
4	*Pseudomonas spp.*	Pink	Bacillus	- Ve	Motile	+Ve	- Ve	- Ve	- Ve	+Ve	+Ve
5	*Salmonella spp.*	Pink	Bacillus	- Ve	Motile	+Ve	+Ve	+Ve	-Ve	- Ve	- Ve
6	*Lactobacillus spp*	Violet	Rod shape	+Ve	Non-Motile	- Ve	- Ve	- Ve	-Ve	- Ve	- Ve
7	*Vibrio spp.*	Pink	Slightly curved	- Ve	Motile	- Ve	- Ve	+Ve	- Ve	+Ve	+Ve

+: Positive,−: negative,

Table 3: Cell density (cfu g^{-1}) of total mesophilic aerobic microorganisms

S. No	Species	Bacterial Density
1	*Mugil cephalus*	2.2×10^{2}
2	*Rastrelliger kanagurta*	1.7×10^{2}
3	*Sardinelle fimbriata*	1.0×10^{3}
4	*Nemipterus japonicas*	1.9×10^{1}
5	*Sepia osculenta*	3.1×10^{2}
6	*Macrobranchium rosenbergii*	4.0×10^{1}
7	*Dasyatis pastinaca*	2.0×10^{3}
8	*Carcharodon carcharias*	1.5×10^{2}
9	*Trichiurus lepturus*	2.8×10^{2}
10	*Litopenaeus vannamei*	3.3×10^{1}

Conclusion

Different pathogenic or potential spoilage bacteria were determined from the current investigation in dried fish, marketed in various locations in the Cuddalore district's coastal village. For future studies, the discovered bacteria should be considered for pathogenic and molecular characterization. Several effective interventions are needed to create a sustainable and profitable industry within the dried fishery sector in Tamilnadu. These include high-quality raw fish for drying, awareness of hygienic and sanitary practices from manufacturing to commercialization, and scientifically reliable and economic dried fish packaging. An effective upstream intervention should also be carried out, such as a market monitoring system to examine organoleptic, chemical, and microbiological food safety.

Reference

1. Azam K, Basher MZ, Ali MY, Asaduzzaman M, Hossain MM. Comparative study of organoleptic, microbiological and biochemical qualities of four selected dried fish in summer and winter. Pakistan J. Biol. Sci,2003:6(24):2030-2033.
2. Ravichandran S, Joseph FS, Kanagalakshmi R, Ramya MS. Variation in Nutritive Composition of Two Commercially Important. International Journal of Zoological Research,2012:8(1):43-51.
3. Azam K. Fisherman community of Kuakata, Bangladesh: Fisheries activities and quality of fish. SUFER Project (DFID-UGC), Khulna University, 2002.
4. Balachandran KK. Post-harvest technology of fish and fish products. Daya Books. Delhi1, 2001, 77-79.
5. Chakrabarti R, Varma PRG. Halotolerant fungi in salted and dried fish at lower Visakhapatnam coast. Fishery Technology,1999:36:28-31.
6. Dhinesh, P., Harikrishnan, S., Kartick, A., Parivallal, M., Ramanathan, T., & Jayalakshmi, S. (2021). Microbial pathogens in canned fish collected from Tamil Nadu. *Int. J. Adv. Res. Biol. Sci*, *8*(9), 23-29.

7. Faruque MO, Nazrul KS, Tonny US, Islam KR, Dey SC, Mona SJ *et al*. Status of an ideal dry fish market of Bangladesh: A study on Asadganj dry fish market, Chittagong. International Journal of Life Sciences Biotechnology and Pharma Research,2012:1(3):214-225.
8. Goswami M, Sathiadhas R, Goswami UC. Market flow, Price structure and fish marketing system in Assam-A case study. CIFE; Mumbai, 2002.
9. Hasan, M. M., Sharma, A., Johnson, F., Mariethoz, G., & Seed, A. (2016). Merging radar and in situ rainfall measurements: An assessment of different combination algorithms. *Water Resources Research*, *52*(10), 8384-8398.
10. Lecocq, T., Hicks, S. P., Van Noten, K., Van Wijk, K., Koelemeijer, P., De Plaen, R. S.,... & Xiao, H. (2020). Global quieting of high-frequency seismic noise due to COVID-19 pandemic lockdown measures. *Science*, *369*(6509), 1338-1343.
11. Daramola JA, Fasakin EA, Adeparusi EO. Changes in physicochemical and sensory characteristics of smoke-dried fish species stored at ambient temperature. African Journal of Food, Agriculture, Nutrition and Development, 2007, 7(6). http://dx.doi.org/10.18697/ajfand.17.1980
12. Hossain MM, Hossain MD, Noor MA, Haque ASMT, Kabir MA. Quality aspects of some dried fish products collected from different super shops of Dhaka city in Bangladesh. Journal of Sylhet Agricultural University,2015:2(2):283-287.
13. Islam MR. Managing diverse land uses in coastal Bangladesh: institutional approaches. Environment and livelihoods in tropical coastal zones, CT Hoanh, TPTuong, JWGrowing and B Hardy (Eds.), CAB International, 2006, 237-248.
14. Nesan, P., Joshua, J., Harikrishnan, S., Parivallal, M., Sudarshan, S., & Jayalakshmi, S. (2021). Fungal flora in different salt cured fishes collected from markets of Cuddalore district, Tamil Nadu. *Int. J. Adv. Multidiscip. Res*, *8*(12), 74-79.
15. Mazumder MSA, Rahman MM, Ahmed ATA, Begum M, Hossain MA. Proximate composition of some small indigenous fish species (SIS) in Bangladesh. International Journal of sustainable crop production,2008:3(4):18-23.
16. Hyun JE, Kim JH, Choi YS, Kim EM, Kim JC, Lee SY. Evaluation of microbial quality of dried foods stored at different relative humidity and temperature, and effect of packaging methods. Journal of Food Safety,2018:38(2):e12433.
17. Balachandran KK. Post-harvest technology of fish and fish products. Daya Publishing House, Delhi,2001:1:77-79.
18. Sanjeev S, Surendran PK. Fate of enterotoxigenic Staphylococci in fish subjected to curing. FisheryTechnology,1996:33:66-68.
19. GOI**.** Handbook of Fisheries Statistics. Ministry of Agriculture. Government of India. New Delhi, 2001.
20. MPEDA.19thIndiaInternationalSeafoodShowatChennaiTradeCentre, Chennai. Press Release–28thFebruary, 2014.
21. **Akh**ouchal I, Haddad MB, Lamine I, Moukrim A, Alla AA. Assessment of the microbiological quality of three frozen seafood products of the Moroccan Atlantic.