IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
June 2019 Vol.:12, Issue:4

Microbiological Assessment of Three Common Species of Bycatch from Visakhapatnam Fishing Harbour

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

T.Sasikala, C.Manjulatha*, DVSN Raju

Department of Zoology, Andhra University, Visakhapatnam.

Submission: 25 May 2019
Accepted: 30 May 2019
Published: 30 June 2019

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Bacteria, Bycatch, Microbiology, Quality, TCC, TPC

ABSTRACT

The present study was carried out for microbiological analysis of three bycatch species in the fishing harbour of Visakhapatnam, east cost of India. The study was performed during the period of Aug 2014 to Jul 2016. The samples were inoculated and counted using standard plate count method and enumeration of total coliforms and fecal coliforms by most probable number method. The microbial count was done by TOTAL BACTERIAL COUNT (TBC) and TOTAL COLIFORM COUNT (TCC) in the fishes of *Trichiurus lepturus, Upeneus vittatus* and *Leiognathus equulus.* The presence of specific fish pathogens *Salmonella* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Vibrio* sp. were investigated. Bacterial infested fish have no or low market value resulting in loss to fishery industry. Hence it is relevant to identify such infectious agents and suggest measures for prevention and elimination of such infections.

INTRODUCTION

Fish is an important dietary component of man. It is important the fish that are consumed should be healthy and free of infection ensuring food safety. An infection, which is caused by viruses, bacteria and parasites among the fishes in natural and man-made culture systems are harmful for fish health and growth and sometimes are very fatal, causing high mortalities. The present study was carried out for microbiological analysis to assess the quality of raw fish sold in the fishing harbour of Visakhapatnam, east coast of India. The study was performed during Aug 2014 to Jul 2016. The aim of the study is to identify the microbial flora and pathogenic bacteria present in the selected bycatch species were *Trichiurus lepturus*, *Upeneus vittatus* and *Leiognathus equulus*. The Visakhapatnam fishing harbour has special yard with modern facilities like ice manufacturing plants, display trays, trained personnel, electric balances and attractive packing system available in harbour for selling and transportation of fish in different areas [1]. Improper hygienic conditions and disposal of waste material favors the adaptability of microbial population to alter. Microbial contamination of the environment may be transferred to the food products directly through surface content by personal, pests, air movements and cleaning regimes [2].

There can be a wide range of microbiota on the skin and gills, and in the intestine, which depends greatly on the microbiological quality of the environment. This can be influenced by the production system, temperature, proximity to settlements and agricultural areas, and fish feeding and harvesting [3 & 4]. While vertical transmission through the egg may be the most common mode of transmission for viruses [5], horizontal transmission from other infected fish, including bycatch is likely [6 & 7]. The direct infection of cultured fish through the consumption of bycatch containing high bacterial loads, particularly of the streptococcal types, is also well-documented [8]. The present authors also reported on diversity of bycatch, organic and inorganic components of bycatch collected from Visakhapatnam fishing harbour [9,10&11].

The quality of fish is based on the physical, chemical and microbiological forms of deterioration are implicated [12].

MATERIALS AND METHODS

In Visakhapatnam fishing harbour, fin fish species of *T. lepturus*, *U.vittatus* and *L.equulus* are fished throughout the year and used of consumption as fresh forms. These fishes are characterized as short lived, fast growing fishes with relatively high rates of natural mortality.

Collection of fish samples

In the present study, fresh fish samples were collected from the fishing harbour during the early hours of the day between 7:00 and 8:00 AM and transferred to lab processed within 1 hour. The fish sample is collected from the fish hold into a sterile aseptic container together with ice. For analysis of microbial populations, above 3 species of commonly edible fish were selected. *T. lepturus, U. vittatus* and *L. equulus* are the fishes that were selected for the present study. Identification of fish was done according to Food and Agriculture Organization (F.A.O) manuals. In the present study, four microbial parameters for examination of sample fishes were considered which includes TOTAL PLATE COUNT (TPC), TOTAL COLIFORM COUNT (TCC) and qualitative analysis of *Staphylococcus* sp., *Pseudomonas* sp., *Salmonella* sp. and *Vibrio* sp.

Preparation of samples

The sample was using the method described by Obi and Krakoviaka. Care was taken to avoid any damage to the specimens. Alimentary canal was cut to avoid damage to parasites. About 10 grams of muscle with skin is swabbed from the dorsal region with a sterile knife. The sample was crushed in a sterile mortar with 10 ml sterile water. From the crushed sample, 1ml of aliquot volume was measured and homogenized in a clean and dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This method is followed for all the 3 fish samples. Each sample was serially diluted and aliquots of each diluted sample were placed on marine agar and incubated at 37^0c for 24 hours.

Microbial count:

An automatic colony counter is used for counting the number of colonies from cultured petri plates and the count was expressed as Colony Forming Unit (cfu/g). The total count and faecal coliform bacteria were enumerated by using the Most Probable Number (MPN) Procedure.

Halophile bacterial count

Halophile bacterial count was determined using 3.5% sodium chloride solution as diluent. Planting was done onto nutrient (plate count) agar with 10% salt plates by spread plate technique. The colonies developed in the planter were counted and expressed as the number of colony forming units/g of sample.

RESULTS

Table-1 & 2 shows the individual results of microbiological analysis conducted on selected 3 samples of fishes. (Table 1 & 2 shows the total bacterial count of the three fish samples). Comparative analysis of TPC and TCC showed great variation from species to species as shown figures 1 & 2. The highest count of TBC was found *T. lepturus* $2.60x10^4$ CFU/g and the lowest count $2.39x10^4$ were found in *U. vittatas.* $2.61x10^4$ were found medium count in *L. equulus.* The bacterial flora on freshly caught fish depends on the environment in which it is caught rather than on the fish species.

The total coliform count as indicator organisms were found in almost all the samples of 3 fishes. The highest count of TCC (160 MPN/g) found in *T. lepturus* and lowest count was found in *U. vittatus*. Almost all values exceed the IAMS limits (100/g) for total coliform and 11/g for faecal coliform that infers on the supply of low quality fish in most of the fish markets. The presence of coliform group *E.coli* is in the higher range, which indicates the contamination of the samples before or during handling processing and marketing.

Table 3 and 4 shows variations of isolates in culture during the study period. In the present analysis *Vibrio* sp., and *Salmonella* sp., were found high range of the population above the selected species. *Pseudomonas* sp., and *Staphylococcus* sp., of pathogens were occurred in low population. The highest range of bacterium found in *T. lepturus* (114 *Vibrio*) and lowest range occurs in *Upeneus vittatas* (11 *Pseudomonas*) similar results represented in table no.3 & 4. And figure 1 & 2. Presence of *Vibrio* sp., the fish can cause infection to the consumer, in the present investigation, *Vibrio* sp., was studied qualitatively and was found in all three fish samples. *Salmonella* sp., is highly pathogenic and this is the major reason for isolation from fish samples. In the present study, *Salmonella* sp., was examined qualitatively and was found in all the three fish samples.

Table –1: Average value of Total plate count of bacteria and total coliform counts in the 3 fish samples in the year 2014-15.

Name of the fish	total bacterial count	total coliform count
Trichiurus lepturus	2.60x 10^4 cfu/g	160 mpn/g
Upeneus vittatus	2.39x10^4 cfu/g	75 mpn/g
Leiognathus equulus	2.58x10^4 cfu/g	93 mpn/g

Table –2: Average value of Total plate count of bacteria and total coliform counts in the 3 fish samples in the year 2015-16.

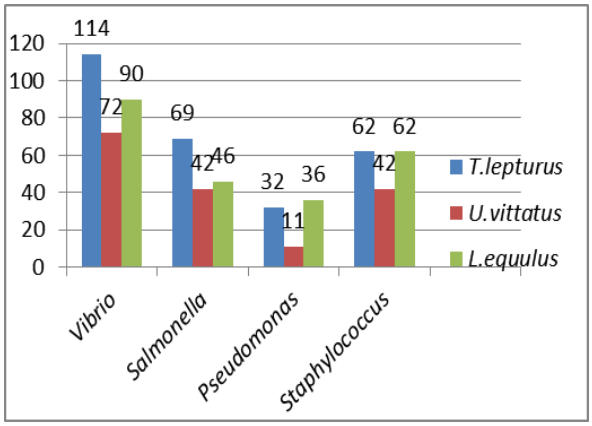
Name of the fish	total bacterial count	total coliform count
Trichiurus lepturus	2.72x 10^4 cfu/g	174 mpn/g
Upeneus vittatus	2.39x10^4 cfu/g	79 mpn/g
Leiognathus equulus	2.61x10^4 cfu/g	104 mpn/g

Table -3: Variants of isolates in the culture during 2014-15

Organisms	*T. lepturus*	*U. vittatus*	*L. equulus*
Vibrio	102	62	80
Salmonella	75	32	50
Pseudomonas	22	10	20
Staphylococcus	52	40	56
Total	251	144	206

Table -4: Variants of isolates in the culture during 2015-16

Organisms	*T. lepturus*	*U. vittatus*	*L. equulus*
Vibrio	114	72	90
Salmonella	69	42	46
Pseudomonas	32	11	36
Staphylococcus	62	42	62
Total	277	167	234



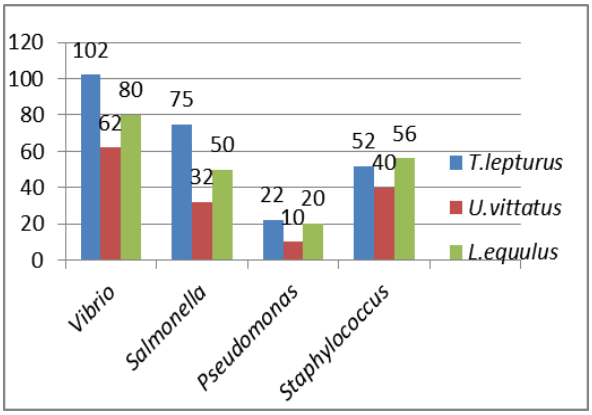


Fig-1 & 2 Variants of isolates in the culture during 2014-15 & 1015-16

DISCUSSION

In the present investigation, the *Vibrio* sp., and *Salmonella* sp., was highly pathogenic bacteria Presence of *Vibrio* sp., in the fish can cause infection to the consumer. In the present investigation, *Vibrio* sp., was studied qualitatively and was found in all the three fish samples. According to a recommendation of the international association of microbiology societies, fresh fish should be free of *Vibrio* (0/gm). The present study revealed that microbial quality was not good due to the presence of *Vibrio* spp. in all the samples. Similar observations were noticed in Visakhapatnam fishing harbour edible marine fish by reference of [13].

With regard to the average value of TPC of bacteria and TCC, it found that highest TPC was found for *T.lepturus* (2.60×10^4 CFU/g) and the lowest count is found for *U.vittatus* (2.39×10^4). The TCC, which is an indicator of organisms were found in all most all the samples of three fishes. The highest count (160 MPN/g) is found in *T.lepturus* and the lowest count (75 MPN/g) is found in *U.vittatus* for the year 2014-15. The same picture can be seen from the analysis carried out for the year 2015-16 with slight variations in the values.

Inhibition tests on solid medium showed that, in general, the majority of fish, bacteria were strongly sensitive to the marine bacteria. Only two strains (*Edwardsiella tarda* and *Pseudomonas aeruginosa*), were resistant to all the antibiotic-producing strains. The results of antagonism assays in sea water, however, varied according to the fish pathogens examined. Experiments conducted using cell-free supernatant fluids of marine bacteria demonstrated the involvement of antibiotic substances in the inhibition of fish pathogens.

Salmonella sp., is high pathogenic and is the major reason for isolation from fish samples. In the present study, *Salmonella* sp., was examined qualitatively and was found in all the three bycatch samples. The results indicate the consequence of contamination of the process, improper handling, hygienic and sanitary conditions of Visakhapatnam harbour. It has been shown that and *Salmonella* sp., can survive for very long periods in tropical waters and once introduced may become adapted to the new conditions favoring the growth of microorganism in the environment. Drinking faecal contaminated water can also lead to an outbreak of the same. Fish harvested from such water can carry *Salmonella* sp. [1 & 2]. The bacterial flora on freshly caught fish depends on the environment in which it is caught rather than on the fish species [3 & 4]. It has been shown that *Escherichia* sp., and *Salmonella* sp., can survive for very long periods in tropical waters and once introduced may become adapted to the new conditions favoring the growth of microorganism in the environment [18].

The presence of *S.aureus* indicates the contamination of the fish and its natural environment by human beings and warm blooded animals. [19] recorded the presence of *S.aureus* in natural micro flora of fin fish and shell fish. This suggests that fish was contaminated with this pathogen during the post-harvest handling procedures [20]. The discussion on the results of bacteriological indices, including a total plate count is, the discussion on changes in quality indices in the present study in relation to review of available related literature. Table-13 shows that there was no any pathogen microorganism such as *Vibrio parahaemolyticus, Escherichia coli, Salmonella,* and *Staphylococcus aureus* in three fish species. And total count in all fish samples was very low in comparison with total counts of Iran standards.

The microbial population present in the natural environment is the source for fish to be polluted [21]. During transportation of contaminated fish to landing centers and wholesale markets, the microorganisms are transferred to the persons involved in the handling process [22]. The quality of fish is based on the chemical and microbiological forms of deterioration are implicated [23].

The quality of fin fish and shell fish is classified into different grades on the basis of their Tri methyl amine nitrogen (TMAN) content according to [24]. The meat containing 0-25 mg percent TMAN as considered as grade 1quality (prime or good), 1-5 mg percent TMAN as grade II quality (acceptable) and the meat having more than 5 mg percent is graded as grade III (spoiled or rejected). [25] States the TMAN limit value standard is 10-15mg/100. [26] recommended 10-15 mg/100mg for human consumption. According to [27] tolerable limit for

TMAN is 12 mg/ 100g. According to [28] tolerable limit for TMAN is 15 mg/100g. According to [29] TMAN limit values for fishery products are good up to 4 mg/400g, scalable up to 10 mg/100g and spoiled up to 12 mg/100g.

Bacterial infested fish have no or low market value resulting in loss to fishery industry. Hence it is relevant to identify such infections agents and suggest measures for prevention and elimination of such infections.

CONCLUSION

The microbial examination of the regard to the average value of total plate count of bacteria and total colony count it found that highest TPC was found for *T.leptutus* (2.60×10^4 CFU/g) and the lowest count is found for *U.vitatus* (2.39×10^4). The TCC, which is an indicator of organisms were found in all most all the samples of three fishes. The highest count (160 MPN/g) is found in *T.leptutus* and the lowest count (75 MPN/g) is found in *U.vittatus* for the year 2014-15. The same picture can be seen from the analysis carried out for the year 2015-16 with slight variations in the values. As far as the variants in the isolates are concerned, it is found that the presence of organism's viz., *Vibrio sp.,* and *Salmonella sp.,* is higher in all the three selected species when compared to the other two organisms viz., *Pseudomonas sp.,* and *Staphylococcus sp.,* of pathogens for the year 2014-15. A more or less similar picture can be seen with slight variation in values for the year 2015-16 also. The overall study has been undertaken to find out to know the quality.

REFERENCES

1. Jagadeesh Chandra Babu.P and Nageswara rao.L (2014). Microbilogical examination of three types of Common Edible Marine Fishes from Visakhapatnam Fishing Harbour, East Coast of India. 7, 289-292.
2. Boyd.RF (1984). General Microbiology. Published by Times Mirror/ Mosby College pp459-461.
3. Reilly.A and Kaferstein.F (1999). Food safety and products from aquaculture. J. Appl Microbiol Symposium Supplement 85:249-257.
4. Hastein.T, Hjeltnes.B,Lillehaug.A, Utne Skare.J, Berntssen.M and Lundebye.A.K (2006). Food safety hazards that occur during the production stage: challenges for farming and the fish industry. Rev Sci Tech Oie 25(2):607-625
5. Mushiake.K, Nishizawa.T, Nakai.T, Furusawa.I and Muroga.K (1994). Control of VNN in striped jack: selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). 29, 177-182.
6. Hedrick.R.P, McDowell.T.S (1995). Properties of irido viruses from ornamental fish. Vet. Res. 26, (5-6), pp. 423-427.
7. Woodland.J.E, Brunner.C.J, Noyes.A.D, Grizzle.J.M (2002). Experimental oral transmission of largemouth bass virus. J. Fish Dis. 25, 669-672.
8. Ghitino.C, Latini.M, Agnetti.F, Panzieri.C, Lauro.L, Ciappelloni.R and Petracca.G (2003). Emerging pathologies in aquaculture: effects on production and food safety.Vet. Res. Commun. 27, 471-479.

9. Sasikala T, Manjulatha C and Raju D.V.S.N. (2019a) Diversity of bycatch at Visakhapatnam fishing harbour; National Journal of Life Sciences ISSN 0972-995X. (Communicated).
10. Sasikala T, Manjulatha C and Raju D.V.S.N. (2019b) Organic composition of three edible bycatch species from Visakhapatnam fishing harbour. International Journal of Zoology Research (Bioinfo publications). ISSN: 2231-3516 & E-ISSN: 2231-3524 (Communicated).
11. Sasikala T, Manjulatha C and Raju D.V.S.N. (2019c); Analysis of Mineral composition in bycatch species from Visakhapatnam Fishing harbour; International journal of life-science scientific research (IJLSSR). (Communicated).
12. Sallam.K.I (2007). Chemica, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. Food Chem., 101 (2): 592-600.
13. Geetha.S, Sri Lakshmi.B, Karuna.Y, Govinda rao.V, Muddula Krishna.N, Ram Sai Reddy. N, Bhavani.K and Ramesh Babu. K (2014). Microbiological examination of three types of common edible marine fishes from Visakhapatnam fishing harbour, east coast of India. 6 (5): 471-474.
14. Fisheries department (2000). Assistant director of fisheries (Western Kenya) annual report.
15. Pelczar, Chan and Krieg, (1993). Microbiology concepts and applications, McGraw-Hill New York. 576-579.
16. Mahmuda.B, Abutweb.A, Monika.D and Sahana.P (2010). A comparative microbiological assessment of five types of selected fishes collected from two different markets, Advances in Biological Research, 4(5): 259-265.
17. Shewan.J.M (1961). The microbiology of sea-water fish. In G. Borgstron (editor), fish as food vol. 1, (Acad. Press Inc, NY). Pp: 487.
18. Fujioka.J.T, Shaw.F.R, Mcfarlane.G.A, Sasaki.T and Bracken.B.E (1988). Description and summary of the Canadian, Japanese and U.S. Joint database of stale fish tag releases and recoveries. NOAA Tech. Memo NMFS F/NWC-137.
19. Clucas.I.J and Ward.A.R (1996). Post-Harvest Fisheries Development: A guide to handling, Preservation, Processing and Quality. 1996 pp.ix + 443 pp.
20. Herrera.F.C, Santos.J.A, Otero.A and Garcia-lopez.M.L (2006). Occurrence of foodborne pathogenic bacteria in retails. Pre- package portions of marine fish in Spain. Journal of applied microbiology, 100(3) 527-536.
21. Chowdhury.M.B.R and Baqluis.M.A (1997). Bacterial flora informed carp (labeo rohota) in Bangladesh. In TW and IH Macrec (eds), Diseases in Asin Aquaculture. III. Fish health section, Asian fish SOC, Manila, pp: 90-94.
22. Das.M.F, Hafiza.M, Ahamed.K and Praveen.S (2007). Microbiological analysis of Some raw fish samples, Bangladesh, Journal of Microbiology, 24(1): 67-69.
23. Rubbi.S.M, Muslemuddin.M. and Wahed.A (1978). The present status of fish Technology and inspection in Bangladesh.
24. Gagon.M. and Feller.C.R (1958). Biochemical methods for determining shrimp quality.1.Study of analytical method. J.Food Tech., 340-345.
25. Schormuller.J (1968). Handbuch derlebensmittelchemie (Band III/I) Berlin Heidelberg-New york: Springer verllag.
26. Connell.J.J and Howgate.P.F (1986). Fish and fish products. In: quality control in the food chemistry (S.M. Herschdoerfer,ed.) pp. 347-405, Academic, London.
27. Stockmer.J, Nieper.L (1984). Parameter zur beurteilung des verderbs von nordsee krabben (Crangan crangan), Archive lebensmittelhygiene.35:5-7.
28. Karnop.G (1988). Histamine in salzsardellen.Archiv furLebensmittelhygiene39: 57-84.
29. Krzymien.M.E and Elias.L (1990). Feasibility study on the determination of fish Freshness by tri methyl amine head-space analysis. J. of Food Sci. vol. 55(5). Pages 1228-1232.