International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Microbial Quality of Assessment of Marine Edible Fishes from Fish Landing Centre, Kasimedu, Chennai, Tamilnadu

Muralidharan Velappan[1], Deecaraman Munuswamy[2]

[1]Research scholar, Department of Biotechnology, AMET University, 135, East coast road, Kanathur-603112, Chennai India

[2]Senior Professor/Dean, Department of Industrial Biotechnology, Dr. M.G.R Educational and Research Institute, Maduravoyal, Chennai-600095, India

Abstract: ***The microbial quality of two common edible marine fishes numbering 25 red snapper (Lutjanus campechanus) and 25 Indian oil sardine (Sardinella longiceps) were collected from fish landing centre, Kasimedu, Chennai was assessed during the period from march 2015 to march 2016. Swabs were taken from skin, gill, oral cavity tissues and intestine were incubated in brain heart infusion broth for 24hrs at 37°C. Differentiation and characterization of various isolates were conducted based on their growth characteristics on specific culture media. The results revealed that the samples were contaminated by seven bacterial species, which includes Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus and Vibrio spp. The skin samples considered the highest isolation of the total isolates. Members of the genus Vibrio spp., such as Vibrio cholera, Vibrio parahaemolyticus and Vibrio vulnificus was the predominant bacterial isolates, and there were considerable numbers of Staphylococcus spp found in all examined samples which were observed in skin, gill, oral cavity tissues and intestine, whereas Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa which were restricted to intestine, gills and oral cavity tissues. The microbial load of all the samples was beyond the acceptable limit which is recommended by FSSAI. This investigation is mainly focus on the pathogenic microbes of these two edible fishes and to create awareness in disease transmission to man.***

Keywords: edible, Vibrio cholerae, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Vibrio vulnificus

1. Introduction

Fish is a source of healthy food for humanity at large section for the world population [1]. Fishing has been a major source of livelihood for coastal and inland fishing communities. The fisheries sector in Tamil Nadu plays a crucial role in the overall economic development. Globally fish resources are of utmost importance for food security and fishes make available 4.3 billion people with about 15% of their animal protein and essential nutrients for growth and maternal health [2].

Despite of all significance as a source of food-related resource for the human welfare marine fish are highly susceptible to a wide variety of bacterial infection [3]. Fish bacterial infections are considered the major cause of mortality in Aquaculture Industry [4]. Moreover we have sparse knowledge about these bacterial species and earlier reports revealed that these microbes are normal in the surface microflora of fish. Recent studies of [5], evinced these bacterial species which causes opportunistic infections to fish. According to the authors [6] found that the evolutionary aspects of fish bacteria and the food-fish harbour unique microbiota are not clearly understood. The investigation was carried out [7] were spoilage of food fish involving microbial species such as *Salmonella, Staphylococcus, Vibrio* spp, and *Escherichia coli,* which in turn dependson their niche environment. In previous research [8] observed, bacterial infections in fish very often precipitated by stress which upsets the defense mechanism against the agents like overcrowding, low Density Oxygen (DO) and also high ammonia content. Studies have demonstrated [9] enteric fever which is caused by the heterotrophic bacteria was not only by consumption of contaminated water, but also the intake of fish and shell fish from contaminated water. Above 80% human infectious diseases which are caused by water-borne diseases like typhoid, cholera and Dysentery, predisposed to such outbreaks of seafood associated diseases are caused by toxic substance of biological origin and inflammatory reactions [10]. Earlier investigations showedthat it was evidently understood the food fish are continuously exposed to microorganisms which are present in water including sewage and fecal contaminations [11].

The main objective of this study was to provide information on the human pathogenic bacteria found in the skin mucous, gills, oral cavity tissues and intestines of these two marine edible fishes that was caught from the fish landing centre.

2. Materials and methods

2.1 Study Area

This study was conducted on two edible fish species *Lutjanuscampechanus, Sardinellalongiceps* collected from Kasimedu fish landing centre, Royapuram coast Chennai.This landing centre has now receives fish arrivals through just five or six large fishing vessels fig1.

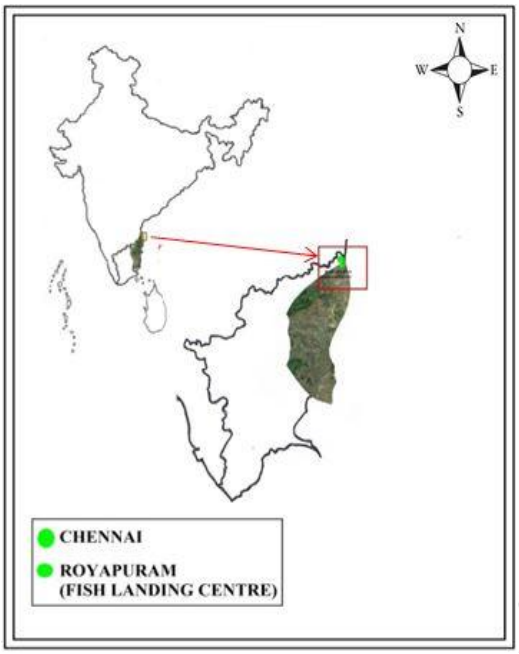

Figure 1: Fish Landing sites Kasimedu, Chennai

Lutjanuscampechanus, this fish has recently recognized as a global fish and one of the most important aquaculture species with different names *Lutjanuscampechanus,* commonly inhabits waters 30 to 200 feet deep [12]. Indian oil sardine scientifically known as *Sardinella* longiceps is a species of ray-finned fish in the genus *sardinella* are coastal, pelagic fish mostly clear saline water in a minimum temperature below 24°C and depth of 350m [13]. It is one of the most commercial fishes in India (unpublished data) (figure 2 A&B).

Figure 2 (a): (Lutjanuscampechanus)

Figure 2 (b): (Sardinellalongiceps)

2.2 Laboratory Analysis

2.2.1 Collection of Fish samples

A total of 50 freshly caught marine edible fish belonging to two genera were examined over a period of twelve months. Samples were collected aseptically in a sterile zip lock plastic bag early in the morning (between 0600 and 0730 hours local time) and transported immediately to the laboratory and processed within three hours of acquisition, and these samples were kept in refrigerator 4-8°C for 24hrs.

2.2.2 Post-mortem and microbiological analysis

Sample preparation was made using the method described by [14]. Each body surface of fish was swabbed with sterile cotton buds which were then used to seed solid Nutrient, MacConkey and Malt Extract Agar (Oxide) plates. For the gut sample, ventral area of fish was disinfected with cotton wool soaked with alcohol were used and the fish was dissected about 1gram of intestine was macerated in 9ml of sterile distilled water. This was serially diluted to$10^{-3}$and from this 1ml was plated using the similar media as skin specimen. The same procedure was applied aseptically to gills and oral cavity tissues. Further all the plates of for the skin specimen numbering 50 plates were incubated at 37°C for 24-48hrs. This was done for all 50 fish samples. The isolates were purified and characterized presumptively by colonial morphology, pigmentation and staining. Added to this, the ability of the isolates to produce oxidase and catalase, ferment lactose, and metabolize glucose fermentatively or oxidatively was tested. Identification of Gram positive species, sucrose and mannitol fermentations and nitrate reductase determination was used. Further, Gram negative isolates were separated on the basis of carbohydrate utilization, production of urease and indole, methyl red, Voges-Proskaeur and citrate utilization reactions. Final identification of these organisms was done by using the phenotypic and biochemical characterization as described by [15].

3. Results

The result of the study have revealed that the freshly caught fishes are infected or contaminated with seven bacterial species isolates from four anatomical sites table 1. The bacterial species were identified for skin mucous are the highest isolation rate of 102(31.57%) of the total bacteria isolates, while 82(25.38%) considered as intestine, 75(23.21%) for oral cavity tissues and 44(13.62%) regarded as gill table 2.The members of the family (*Enterobacteriaceae)*such as *Escherichiacoli, Salmonella typhimurium, Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus,* were the common bacterial isolates identified for (50%) and also genera of Gram positive bacteria *staphylococcus* were found in all tissues whereas the Gram negative, coliform bacteria *Escherichia coli* which is limited in extent to the intestinal tract. *Escherichia coli* were isolated only from the skin mucous and gills. The presence of *Salmonella* sppwas not colonized in the gill and intestine of both the fishes.

Table 1: Frequency of isolation ofbacterial species from different anatomical sites

Bacteria Species	*Skin*	*Gill*	*Oral Cavity Tissue*	*Intestine*	*Total*	*Percentage*
E. coli	9	10	8	-	27	8.3 %
S. typhimurium	5	-	3	-	8	2.4 %
P. aeruginosa	7	5	-	7	19	5.8 %
S. aereus	20	9	10	6	36	11.1 %
V. cholera	15	12	16	28	71	21.9 %
V.vulnificus	20	18	18	22	78	24.1 %
V. parahaemolyticus	25	20	20	19	84	26.9 %
Total	102	44	75	82	32	99.6 %

Table 2: Percentage isolation from the anatomical sites

Anatomical Sites	*No.of Isolates*	*Percentage of Isolates*
Skin	102	31.57
Gill	44	13.62
O.cavity tissue	75	23.21
Intestines	82	25.38
Total	303	93.78

Whereas members of the genus *Pseudomonas* was isolated from skin mucous, gill and intestine.

The Gram negative, fermentative bacteria (such as *vibrionacae*) *V. parahaemolyticus* (26.0%) and *V. vulnificus* (24%) are the highest colonization in all tissues of two fishes were examined.There was no significant differences were showed in the frequencyof isolation of the other bacterial species from the other four sites. And there were no fungal species recovered from the four anatomical sites.

4. Discussion

In the present investigation here we confirmed that fish can be infected with a variety of microbial species especially those of bacteria in the marine environment. This findings substantiates with the findings of [16] reported that the quality of water from where the fishes were caught and also the sanitary condition of the landing centres. It has also been established that the quality of fishes and their spoilage are the severe concern for human health [17].The human pathogen from four anatomical sites (skin mucous, gill, oral cavity tissues and intestine) of two marine edible fishes were isolated and identified the occurrence seven species such as *Escherichia coli*, *Salmonellatyphimurium, Vibriocholerae, Vibriovulnificus, Vibrioparahaemolyticus, Staphylococcus aereus* and *Pseudomonas aeruginosa*are mostly causes food-borne diseases[18]. From this study on these two genera of fishes the skin mucous, gills, oral cavity tissues and intestine were heavily loaded with different *Vibrio* species,however, most of the isolates identified as *Vibrio parahaemolyticus.*We found evidence from the literature [19] detected halophile bacterium *Vibrio parahaemolyticus* from (Sangara-Red snapper, *Lutjanuscompachanus*strains through PCR method from the coastal area of cuddalore district. According to author [20] reports 25% of food-borne disease was caused by *vibrioparahaemolyticus* when compare to other *vibrio* species.*Vibrio vulnificus,* one of the isolates which indicate as reported by [21] hygienic quality and freshness of fish which was seen decreasing in summer season. Another report from [22] found this Gram-negative bacterium was the major cause of seafood-related deaths especially immuno- compromised hosts. The present Investigation of isolation of *Vibrio* spp., agreed with the findings of [23] isolated on another species of fish such as Seabass (*Dicentrarchus labrax L*) and Seabream (*Sparus aurata L*) among the bacterial species *Vibrio* spp., are the highest count. Presence of bacterial species such as *Pseudomonas aeruginosa* in skin and gills of freshly caught fishes depends on the microbial contents of the water in which the fish live [24]. The occurrence of isolates from the gill and the skin mucous can be accounted for mainly by the filtereffect of the former or the slime layer of the former and partly as a result of the active bacterial multiplication and adaptation was described by [25]. Studies were conducted in the isolates identified as members of Enterobacteriaceae, particularly coliform group *Escherichia coli*, which indicates the contamination of the samples before or during handling processing and marketing [26]. Therefore, the isolates potentiate serious consequences to their hosts (fishes) to the animals that feed on them and finally to man. The microbial population constitutes a significant burden throughout the lifespan of fishes as it has role in nutrition, growth and disease susceptibility [27], for example many outbreaks of *Salmonellosis* have been attributed to livestock feeds, and though fish products prepared for human consumption appear to be less of a problem than animal feeds, which are certainly of more immediate public health concern. Reports of [28] that in *Escherichia coli, Salmonella* spp*.,* can survive for very long periods in tropical waters and once introduced may become adaptable to the new conditions favoring the growth of microorganisms in the environment. The presence of *Staphylococcus* species is not a natural micro-flora in fishes including shellfish which was observed by [29]. The enterotoxins produced by *Staphylococcus aureus* isa major public health importance because it has the ability to make several types of toxins, many of which are responsible for food poisoning which causes gastroenteritis after consumption of fish products.The economic and public health problems are further compounded by the development of antibiotic resistance among the microflora isolated; particularly the enteric bacteria [30].It is therefore greater attention must be given to the microflora of edible fishes particularly fish landing sites. Surveillance of potential contaminant bacteria in marine edible fish is crucial for sustenance of public health. It is obvious from the study that the bacterial quality of fish from fish landing site is poor and this causes a serious public health problem.

5. Conclusion

The problem arises, concerning the quality and safety right from the beginning to an attempt that is fish catching, microbial load in fish initially sets in due to high level of contaminated water which allows further unhygienic handling done by the fishermen and also it passes through a long custody before it arrives eventually to the consumer.

The present study of large populations of these pathogenic bacteria indicates high level of fecal contamination at the landing centre was found to be higher than the approved safety standard by Food Safety and Standards Authority of India (FSSAI).To control this situation it is imperative to follow the code of practice in connection with handling, icing, disposal system, storage including hygienic measures.

Hence the microbial quality of marine fishes suggest carefulness and sufficient precaution for handling the food fishes thereby preventing contamination of serious bacterial pathogen like *Escherichia coli*, *Salmonella typhimurium, Vibrio* spp, *Staphylococcus aereus* which causes typhoid, cholera. This study further strengthens the reports of possible microbial contamination.

6. Acknowledgements

The author immensely acknowledge for the constant support, encouragement, constructive suggestions to the

following1.Prof.Dr. M. Deecaraman 2.my wife 3.And my beloved son. The author also acknowledge Royal biotech Pvt Ltd,Velachery, Chennai for the great support to given during Laboratory involvement to complete this research article.

References

[1] Velmurugan, S., Shani John, T., Sheta Alemu. And SenthilNagaraj, 2015. Determination of microbial contamination in Parangipettai coastal water fish during handling. Journal of Microbiology and Biomedical Research.1(5): 2395-5678.
[2] CFI, 2016. Global Coastal Fisheries Initiative Program, Global Environment Facility News.
[3] Petronillah,R., Sichewo, Robert Gono, K., John Muzvondiwa, V., Nyoni Sizanobuhle, 2013. Isolation and identification of pathogenic bacteria in edible fish: A CaseStudy of Fletcher Dam in Gweru, Zimbabwe.International journal of Science and Research.9(2):2319-7064.
[4] Madhuri Sharma, A.B., Shrivastav, Y.P., Sahni and GovindPandey. 2012.Overview of the treatment and control of common fish disease.InternationalResearch Journal of Pharmacy. 3(7): 2230-8407.
[5] PilarGonzalez- parraga, Alberto Cuesta, Meseguer,J., and Angles Esteban, M. 2011. Marinemicroorganisms: the world also changes; communicating current research and technologicaladvances, FORMATEX.
[6] AndreaLarsen,ZhenTao,Stephen,A.,BullardandCovadonga, R., Arias. 2013.Diversity of the skinmicrobiota of fishes: evidence for host speciesspecificity, Federation of European MicrobiologicalSocieties. 85: 483-494.
[7] SubashWalke, ShivajiKashte, NavnathParwe, ShivajiChavan. 2015.Opportunistic Pathogens in the Mucous Of Skin, Fins, and Mouth of LabeoRohita, International Journal of Fisheries and Aquatic Studies.3(1):169-172.
[8] Khatun, H.,Hossain, MD., Jahan, SN., and Khanon,DA. 2011. Bacterial Infestation in Different Fish at Rajshahi, Journal of Science Foundation. 9(1-2): 77-84.
[9] Romero Jarero Jorge, PilarNegrete Redondo and Maria del Carmen MonroyDosta.2016. International Journal of Aquatic Science,7(1):pp 6-12, 2008-8019.
[10] Sujatha, K., Senthilkumar, S., Sangeetha and Gopalakrishnan. 2011. Isolation of human pathogenic bacteria in two edible fishes, Priacanthushamrur and Megalaspiscordyle at Royapuram waters of Chennai, India, Indian journal of Science and Technology, 4(5): 0974-6846.
[11] Austin. B, 2002.The Bacterial Microflora of Fish, Revised, The Scientific World JOURNAL, 2, 1537-744X.
[12] Jayabarath, JenyJoseline and Gobika, 2015. Isolation & Characterization of Antibiotic resistant bacteria from Lutjanuscampechanus, World Journal of Pharmaceutical Research, 4(8):2277-7105.
[13] Nyarko, H.D., Obadai, E.A., Boamponsem, L.K.,Coomson, S.S and Aniwe, Y.2011. Microbial profile of smoked sardine *(Sardilellaaurita)* AT smoking sites and market centres of Tema, Ghana-1, Scholars Research Library, 3(3):443-453.
[14] RitaR. colwell and John Liston.1962.Bacterial Flora of Seven Species of Fish Collected at Rongelapand Eniwetok Atolls, Scholars Space,16(3).
[15] Olayemi, Adedayo and Ojo. 1990. Microbiological studies on fresh water fishes from the ASA River, Ilorin, Nigeria, Journal of Aquaculture in the tropics. 5(2):135-139.
[16] ChandravalDutta, ChandranSengupta. 2016. Prevalence of Escherichia coli in Fish and Shrimps obtained from Retail Fish markets in & around Kolkata, India, Frontiers in Environmental Microbiology. 2(1):5.
[17] SubhashWalke,ShivajiKashte, NavnathParwe, ShivajiChavan. 2015. Opportunistic pathogens in the mucous of skin, gills, fins and mouth of LabeoRohita, International Journal of Aquatic Studies, 3(1): 169-172.
[18] Petronillah R., Sichewo, Robert Gono, K., John Muzvondiwa, V., NyoniSizanobuhle, 2013.Isolation and Identification of pathogenic bacteria in edible fish: A case study of Fletcher dam in Gweru, Zimbabwe, International Journal of Science and Research, 2(9): 2319-7064.
[19] Elamparithi, P., and Ramanathan. N. 2011. Identification of Vibrio parahaemolyticus Isolates by PCR Targeted to the toxR Gene, International Journal of Pharmacaetical&Biological Archives.2(6):1735-1737.
[20] Feldhusen, F. 2000. The role of seafood in bacterial food-borne diseases.*Microbes and infection,* 2:1651-1660.
[21] Maysoon Sabah Abbas., Isolation of bacteria from fish, 2014.Internaional journal of Advanced research, 2 (3): 274-279.
[22] NaielBisharat, Daniel, I. Cohen, Martin C. Maiden, Derrick W. Crook, Tim Peto, Rosalind M., Harding, 2007. The evolution of genetic structure in the marine pathogen, *Vibrio vulnificus*, Science Direct, Infection, Genetics and Evolution, 7: 685-693
[23] Tanekhy,2013. Some study on bacterial infection in some cultured marine fish, Journal of the Arabian Aquaculture Society, 8(1).
[24] Ghaly, A.E., Dave, D., Budge,S.,and Brooks, M.S., 2010. Fish Spoilage Mechanisms and Preservation Techniques: Review, American Journal of Applied Sciences, 7(7):859-877.
[25] Olayemi,AdedayoandOjo,1990.Microbiological studies on fresh water fishes from the ASA River, Ilorin, Nigeria, Journal of Aquaculture in the Tropics,(5):135-139.
[26] Geetha, S., Sri Lakshmi, B., Karuna, Y., GovindaRao, 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, World Journal of fish and Marine Sciences, 6(5): 471- 474.
[27] Trust and Sparrow, 1974.The bacterial flora in the alimentary tract of fresh water Salmonid fishes, Canadian Journal of Microbiology, 20(9): 1219- 28.
[28] Geetha, S., Sri Lakshmi, B., Karuna, Y., GovindaRao, 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, World Journal of fish and Marine Sciences, 6(5): 471- 474.
[29] Salihu,M.D., Junaidu, A.U., Magaji, A.A., Faleke, O.O., Yusuf, Y., Abubakar, M.B., Tambuwal, F.M and Samaila, S, Bacteriological quality of fresh water fishes caught from Sokoto River, Nigeria, Journal of Veterinary Advances, 2(1):pp65-69.
[30] Olayemi,Adedayo and Ojo,1990.Microbiological studies on fresh water fishes from the ASA River, Ilorin, Nigeria, Journal of Aquaculture in the Tropics.(5):135-139.

Author Profile

V. Muralidharan BSc. Clinical microbiology from Punjab Technical University, Jalandhar, Punjab during 2007-2010. MSc., microbiology from Vinayaga mission University, Salem, Tamil Nadu during 2011-2013 and pursuing PhD (part-time) in Biotechnology from AMET University, East Coast Road, Kanathur, Chennai from 2014 onwards.

Dr.M.Deecaraman, MSc. MPhil., PhD (Tulane,New Orleans, USA).Present Position: Senior Professor / Deanat Dr. M.G.R Educational &Research Institute, Maduravoyal, Chennai-600095. Specialization: Reproductive physiology of invertebrates/ endocrinology, Animal biotechnology/Aquaculture/ Environmental toxicology, Photochemistry produced till date 25 PhD candidates at the University of Madras& M.G.R Research Institute, Maduravoyal, Chennai- 600095. Recently awarded Lifetime award teaching & research (more than 40 years Annamalai Unversity- Tamil nadu).