Identification of Potentially Pathogenic Bacteria from Three Species of Fish at Mukkombu Region of the River Cauvery, Tamil Nadu, India

R. Sivakami and P. Balasubramanian

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 06

Res. Jr. of Agril. Sci. (2022) 13: 1869–1872

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2022)
13(6): 1869–1872

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Short Communication

Identification of Potentially Pathogenic Bacteria from Three Species of Fish at Mukkombu Region of the River Cauvery, Tamil Nadu, India

R. Sivakami*[1] and P. Balasubramanian[2]

Received: 07 Oct 2022 | Revised accepted: 26 Nov 2022 | Published online: 20 Dec 2022

***Key words*:** Pathogenic microbes, Freshwater fishes, Organs of fishes, Total plate count

India with its exploding human population will have to utilize all its avenues for increasing its food production [1]. Hence, thrust is now being made to use all the aquatic systems for aquaculture. However, pathogenic microbes present in these systems can be a potential threat to the handlers and people consuming these organisms. Hence increased attention is now given to the possibility of fish acting as vectors of human pathogenic bacteria [2-5]. Kvenberg [6], Rodricks [7] classified pathogens associated with fish as indigenous and non-indigenous. The non-indigenous contaminate the fish or habitat one way or the other while the indigenous pathogens are found living naturally in the fish habitat [8].

According to Novotny *et al.* [9] human infectious caused by pathogens transmitted from fish or the aquatic environments are quite common and depend on the season, patients contact with the fish and related environment, dietary habits and the immune status of the individual. Further, Sichewo *et al.* [8] suggested that great economic losses had to be incurred due to diseases like dysentery and diarrhea resulting from consumption of contaminated fish. Hence, it was thought worthwhile to investigate the occurrence of bacterial pathogens in three species of fishes from the Cauvery River at Mukkombu in Tiruchirappalli District of Tamil Nadu, India.

Site of collection

For the present investigation, the samples were collected from the River Cauvery, Upper Anicut, Trichy District, Tamil Nadu. This river has a rich source of fish diversity with a variety of fishes like fin fishes and shellfishes.

* **R. Sivakami**
✉ drsiva17@gmail.com

[1] PG & Research Department of Zoology, Arignar Anna Government Arts College, Musiri, Tiruchirappalli - 621 211, Tamil Nadu, India

[2] PG & Research Department of Zoology, Government Arts college (Autonomous), Kumbakonam - 612 002, Tamil Nadu, India

Collection of samples

Samples of water soil, fishes were collected from Upper Anaicut in 2022. The water samples were taken in plastic containers kept in an ice box and brought to the laboratory. Fishes were collected in containers and brought to the laboratory. The weight and length of the fishes were recorded. The fish were dissected, and the tissues like skin and intestinal tracts were taken separately.

Bacterial analysis and its identification

Water samples for microbiological analyses were collected, put aseptically into sterile 500 ml sampling bottles and examined within 1-2 hours of collection in the laboratory. All water samples were analyzed for the presence of total and faecal coliform bacteria, faecal *Streptococci* and pathogenic *Salmonella* by the most probable number (MPN) method following the American Public Health Association [10] procedures. The Total Viable Count (TVC) of all heterotrophic bacteria was done on nutrient agar plates incubated at 28 °C for 48 hours.

Ten specimens from each fish species were examined on the day of harvest. Swab samples of about 4 – 5 cm^2 fish skin area were collected and inoculated onto media as those used for the water samples to estimate the MPN values. Pieces of fish skin, gill, mouth and digestive tract were collected separately under aseptic conditions and put into sterile Petri dishes. Corresponding organs from the same fish species were pooled, weighed and homogenized with a sterile warring blender with 10 ml of 0.1% phosphate buffer saline of pH 7.5 per gram of fish tissue. A volume of 0.1ml of the homogenate was plated subsequently onto nutrient agar and Mac Conkey agar and incubated at 37 °C for 24 - 48 hrs. For qualitative identification of various bacteria from water and fish samples, fresh solid media of modified fecal coliform (M-FC) agar were inoculated in duplicate and incubated at 37 °C for 24 hours. After distinct coloured colonies of various bacteria developed on the plate, the identification of the bacterial colonies were done according to Edwards and Ewing [11], Cowan [12], Martin and Washington [13], Brenner [14] and Cheesbrough [15].

The results of the Total Plate Count (TPC) analyzed in the various organs of the three fish species are presented in (Table 1). As evident from the table, the TPC was found to vary from 12.0 to 28.4×10^7 cfu/gm in the various species. While the lowest TPC was recorded in the intestine of *Labeo ariza*, the highest TPC was noticed in the skin of *Etroplus suratensis*. A comparison of the TPC levels in the various organs assessed reveals that TPC was lowest in intestine followed by gill, mouth and skin in all the fishes examined. The coliform count varied from 8.3×10^6 cfu/g to 17.8×10^6 cfu/g. While the minimum count recorded in the intestine *Puntius amphibious*, the maximum count was recorded in the mouth of *E. suratensis*. In general, the coliform count was minimal in intestine, followed by gill, skin and mouth in ascending order in all the three species of fish.

Table 1 Pathogenic bacteria isolated from three species of fish organs and water at Mukkumboo, the River Cauvery

Details of the Species	Unit		*Puntius amphibious* (Fish I)				*Labeo ariza* (Fish II)				*Etroplus suratensis* (Fish III)				Water
			Intestine	Gill	Skin	Mouth	Intestine	Gill	Skin	Mouth	Intestine	Gill	Skin	Mouth	
TPC	Cfu/g	×10^7	19.2	19.5	24.2	22.3	12.0	15.6	20.2	16.2	20.5	23.0	28.4	24.6	28.0
Coliforms	Cfu/g	×10^6	8.3	10.2	17.4	17.6	10.4	12.4	15.2	16.2	14.5	15.4	17.4	16.8	16.5
Pseudomonas aerginosa	Cfu/g	×10^5	3.6	4.6	8.2	7.6	5.6	6.5	7.2	5.6	8.0	8.6	9.6	9.0	10.2
Vibrio Cholera	Cfu/g	×10^5	2.2	4.4	7.6	5.2	5.0	5.2	6.2	4.3	6.4	7.8	8.4	8.2	9.2
Escherichia coli	Cfu/g	×10^5	3.6	3.9	4.5	4.2	2.8	3.0	3.6	3.1	3.9	4.8	5.4	5.2	6.2
Staphylococcus aureus	Cfu/g	×10^5	7.6	8.9	14.4	12.6	7.4	9.4	11.2	10.4	12.4	13.8	14.8	14.0	14.5
Shigilla dysentariae	Cfu/g	×10^4	3.6	4.2	9.6	8.4	6.4	7.4	8.2	7.5	10.6	11.2	12.2	11.4	12.5
Salmonella typi	Cfu/g	×10^4	8.2	9.4	12.6	10.2	7.8	8.6	11.2	10.0	11.2	12.5	14.5	13.6	14.5
Enterococcus faecalis	Cfu/g	×10^4	9.4	10.2	13.6	11.2	8.4	10.2	12.6	10.4	12.0	13.6	16.3	15.6	16.2
Aeromronas hydrophila	Cfu/g	×10^4	10.6	12.4	17.6	13.6	11.2	13.2	15.6	13.0	13.8	15.2	18.6	17.6	18.4
Actinobacter Calcoaceticus	Cfu/g	×10^5	7.2	13.2	18.2	15.6	-	-	-	-	15.2	16.0	19.2	16.8	17.6
Flavobacterium branchiophilum	Cfu/g	×10^2	-	-	-	-	-	-	-	-	2.0	2.1	2.8	2.4	3.4
Enterobacter aerogenes	Cfu/g	×10^3	4.2	9.2	14.2	11.2	6.4	7.6	9.6	9.4	12.0	13.8	15.2	14.2	16.2
Closterium botulinum	Cfu/g	×10^3	1.0	1.4	2.3	2.0	--	--	--	--	3.4	3.8	4.2	3.9	4.9
Proteus vulgaris	Cfu/g	×10^4	3.6	2.0	4.7	4.0	2.1	3.0	3.6	3.2	4.0	6.3	5.6	5.2	6.4

Pseudomonas aeruginosa was recorded in all the organs of all the three fishes studied. Nevertheless, it was found to vary from 3.6×10^5 cfu/gm (intestine of *P. amphibious*) to 9.6×10^5 cfu/gm (skin of *Etroplus suratensis*). In general, the lowest count was recorded in the intestine followed by gills, mouth and skin in ascending order in all the fishes analyzed *Vibrio cholera* counts on the other hand was found to range from 2.3×10^5 Cfu/gm (intestine of *P. amphibious*) to 8.4×10^5 cfu/gm (skin of *Etroplus suratensis*). Here also, the lowest *V. cholerae* count was recorded in the intestine followed by gill, mouth and skin in ascending order in all the fishes examined. *Escherichia coli* counts varied between 2.8×10^5 cfu/gm in the intestine of *L. ariza* and 8.4×10^5 cfu/gm in the skin of *Etroplus suratensis*. The minimal counts were noticed in intestine followed by gills, mouth and skin in all the fishes.

The minimum count of *Staphylococcus aureus* was noticed in the intestine of *L. ariza* (7.4×10^5 cfu/gm) and the maximum in the skin of *Etroplus suratensis* (14.8×10^5 cfu/gm) *Shigilla dysentariae*, on the other hand, recorded its minimal count in the intestine of *P. amphibious* (3.6×10^4 cfu/gm) and the maximum count in the skin of *Etroplus suratensis* (12.2×10^4 cfu/gm). Here also, the minimal counts were recorded in the intestine and the maximum in the skin in all the 3 fishes.

Salmonella typhi on the other hand was found to range from 7.8×10^4 cfu/g (intestine *L. ariza*) to 14.5×10^4 cfu/gm (skin *Etroplus suratensis*). Among the various organs, the lowest count was found in intestine followed by gills, mouth and skin uniformly in all the fishes *Enterococcus faecalis* ranged from 8.4×10^5 cfu/gm to 16.3×10^5 cfu/g in the various organs. The lowest level was recorded in intestine followed by gill, mouth and skin uniformly in all the fishes *Aeromonas hydrophila* counts varied between 10.6 and 16.3×10^4 cful/gm. The lowest levels were again recorded in the intestine and the highest in skin in all the three fishes.

Enterobacter aerogenes count ranged between 4.2×10^3 cfu/gm in the intestine of *P. amphibious* and 15.2×10^3 cfu/gm in the skin of *Etroplus suratensis*. In general, among the various organs, intestine recorded the least count followed by gill, mouth and skin in all the 3 fishes. *Proteus vulgaris* also recorded the same trend with the lowest count being 1.6×10^4 cfu/gm in the intestine of *P. amphibious* and the maximum in the skin of *E. suratensis* (5.6×10^4 cfu/gm).

Actinobacter calcoaceticus was recorded in *P. amphibious* and *Etroplus suratensis* and was absent in *L. ariza*. Then count ranged between 7.2×10^5 cfu/gm in the intestine of *P. amphibious* and 19.2×10^5 cfu/gm in the skin of *Etroplus suratensis*. However, among the various organs, intestine recorded the lowest levels followed by gill, mouth and skin in both the fishes. *Closterium botulinum* also was recorded only in *P. amphibious* and *Etroplus suratensis* and absent in *L. ariza*. Among the various organs, the lowest count was noticed in the intestine of *P. amphibious* and the highest count in the skin of *Etroplus suratensis*. Here again, in both the fishes, the lowest count was recorded in intestine followed by gills, mouth and skin respectively. *Flavobacterium branchiophilum* was recorded only in *Etroplus suratensis* and was not found in the organs of *P. amphibious* and *L. ariza*. Nevertheless, the count, in various organs appeared to follow the pattern of other microbes in that the minimum load was recorded in the intestine followed by gills, mouth and skin in ascending order.

A perusal of literature reveals that most of the bacteria recorded on the various organs of the three fishes have also been reported by others in various species of fish [16-21]. The bacterial composition in all the fish species appeared to be a reflection of the bacterial composition found in the system several authors have also reported that the bacterial flora of fish is a reflection of their respective environments [22-25].

In the present study, a total of 14 bacterial species could be identified. According to Sickewo *et al.* [8], the diversity of potential pathogens from the samples of fish is a concern. This is all the more true today, because most of the people in the world have been immuno compromised due to the recent COVID pandemic. Further, the present of many pathogens in all the fish analyzed is a health concern as they can cause various diseases in man like food poisoning, diarrhea, typhoid, shigellosis, etc. Claucas and Ward [26] reported that pathogens like *S. aureus, Salmonella, Shigella* and *Pseudomonas*, when present in food are likely to cause food borne diseases. As all these pathogens have been isolated in all the three species of fish, the chances of food borne diseases appear to be high.

In the present analysis, microbes like S. *typhi, S. aureus S. dysentariae* and *E. coli* could be identified. Sichewo *et al*. [8] the above microbes are indigenous pathogens that contaminate the fish or fish habitats in one way or the other. Further, the presence of *Salmonella, Shigella* and *E. coli* clearly indicates the presence of faecal and environmental pollution as already suggested by Yagoub and Ahmed [27-28]. Sichewo *et al*. [8] also suggested that *E. coli* is recognized as a reliable indicator of faecal contamination in small numbers and in large numbers it indicates mishandling.

Thus, the present study, clearly indicates the presence of potentially pathogenic disease-causing microbes in all the fishes analyzed. Generally, the presence of pathogens in the fishes indicates a reflection of their respective environments they live this could be the only reason as to why bacteria like *A. calcoaceticus* and *C. botulinum* were recorded in *P. amphibious* and *E. suratensis* and not in *L. ariza*. This suggests that the microhabitat of *E. suratensis* and *P. amphibious* were more similar than that of *L. ariza*.

Nevertheless, it is clear that stringent laws and enhanced monitoring along with food and safety training to both fisher folk and handless along with consumers on various aspects of Good Hygiene Practice (GHP), Good manufacturing practice (GMP) and HACCP as suggested by Sichewo *et al*. [8] is the need of the honour. Thus, will create awareness to the public at large which to a large extent can prevent food borne infections.

SUMMARY

To feed the exploding human populations, aquaculture has been suggested as a way to provide cheap protein on a large scale. However, of late, microbial examination of fish organs have revealed the presence of potential disease-causing bacteria. Hence a study was done to assess the bacterial composition in the various organs of three species of fish living in the Cauvery River from Mukkombu region of Tiruchirappalli, India. Study revealed the presence of 14 bacterial species. There were potentially disease-causing microbes like *Vibrio, Salmonella, Shigella, E. coli, Enterococcus* etc. The presence of their pathogens indicates the need for passing string out laws as well as creating awareness so that fish borne diseases can be controlled. A notable feature recorded in the present study was that the skin recorded the highest bacterial load in all the three fishes examined.

LITERATURE CITED

1. Premkishore G, Sivakami R, Chandran MR. 1999. Isolation of potent human bacterial pathogens from carps reared in a rock poll in Tamil Nadu. *India Journal of Bioscience* 10: 28-40.
2. Hejkal TW. 1983. Bacteriological, virological and chemical evaluation of wastewater– aquaculture system. *Water Research* 16: 120-130.
3. Sivakami R, Premkiskore G, Chandran MR. 1996. Occurrence and distribution of potentially pathogenic Enterobacteriaceae in carps and pond water in Tamil Nadu. *India Aquaculture Research* 27: 375-378.
4. Reichenback-Klinke HH. 1973. *Fish Pathology*. TFH Publications, Neptune City, New York.
5. Mhango M, Mpuchane SF, Gashe BA. 2010. Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. *African Journal of Food, Agriculture, Nutrition and Development* 10(10): 4202-4218.
6. Kvenberg EJ. 1991. *Non-indigenous Bacterial Pathogen*. *In*: (Eds) Microbiology of Marine Food Products. Donn R., Wand Cameron, H., Van Nostrand Reinhold, New York. pp 263-291.
7. Rodricks EG. 1991. *Indigenous Pathogen: Vibrionaceae of Microbiology of Marine Food Products*. Reinhold, New York. pp 285-295.
8. Sichewo RK, Gono JV, Muzvondiwa NS. 2013. Isolation and identification of pathogenic bacteria in edible fish: A case study of Fletcher Dam in Gweru, Zimbabwe Petronillah. *International Journal of Science and Research* 3(11): 897-904.
9. Novotny L, Dvorska L, Lorencova A, Beran V, Pavlik I. 2004. Fish: A potential source of bacterial pathogens for human beings. *Veterinarni Medicina* 49: 343-358.
10. APHA. 2000. *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. American Public Health Association, Washington, USA. pp 2240.
11. Edwards PR, Ewing WA. 1972. *Identification of Enterobacteriaceae*. 3rd Edition. Burgess Publishing, MN. pp 362.
12. Cowan ST. 1974. *Cowan and Stell's Manual for the Identification of Medical Bacteria*. 2nd Edition. Cambridge University Press, Cambridge. pp 103-111.
13. Martin WJ, Washington JA. II. 1980. *Enterobacteriaceae*. In the Manual of Clinical Microbilogy, 3rd Edition. (Eds) E. H. Lennette, A Belows, W.J. Hausler & J.P. Truant), American Society of Microbiology, Washington, DC. pp 195-219.
14. Brenner DJ. 1984. *Enterobacteriaceae*. *In*: Bergey's Manual of systematic Bacteriology. Vol. 1. (Eds. N. R. Kreig), Williams and Wilkins, Baltimore, M. D. pp 408-516.
15. Cheesbrough M. 1989. *Medical Laboratory Manual for Tropical Countries*. Vol. II, Microbiology, Butterworth Heinemann, London. pp 262.

16. Janssen WA. 1970. Fish as potential vectors of human bacterial diseases of fishes and shellfishes. *American Fisheries Society Special Publication* 5: 589-607.
17. Souter BW, Sonstegard RA, Mc Dermott LA. 1976. Enteribacteria in carp (*Cyprinus carpio*) and white suckers (*Catostomus commersoni*). *Journal of the Fisheries Research Board of Canada* 33: 1401-1403.
18. Fasanya OOA, Oladimeji AA, Yakubu UJ. 1988. Bacterial microflora associated with the skin and gills of *Tilapia nilotica* (*Oreochromis nulotica*). *Nigerian Journal of Applied Fisheries and Hydrobiology* 3: 49-50.
19. Lawton RL, Morse EV. 1980. *Salmonellae* survival in freshwater and experimental infection in goldfish (*Carassius auratus*). *Journal of Environmental Science and Health* 15(4): 339-358.
20. Ogbondeminu FS, Okoye FC. 1992. Microbiological evaluation of an untreated domestic waste water aquaculture system. *Journal of Aquaculture in the Tropics* 7: 27-34.
21. Abisoye BF, Ojo SKS, Adeyemi RS, Olajuyigbe OO. 2011. Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. *Prime Journal of Microbiology Research* 1(2): 23-26.
22. Geldreich EE, Clarke NA. 1966. Bacterial pollution indicators in the intestinal tract of freshwater fish. *Applied Microbiology* 14: 429-437.
23. Nieto TP, Toranzo AE, Barja JL. 1984. Comparison between the bacterial microflora associated with fingerlings of rainbow trout cultured in two different hatcheries in North West of Spain. *Aquaculture* 42: 193-206.
24. Buras N, Duek L, Niv S, Hepher B, Sandbank E. 1987. Microbiological aspects of fish grown in treated waste water. *Water Research* 21: 1-10.
25. Ogbondeminu FS. 1993. The occurrence and distribution of enteric bacteria in fish and water of tropical aquaculture ponds in Nigeria. *Journal of Aquaculture in the Tropics* 8: 61-66.
26. Claucas IJ, Ward AR. 1996. Post-harvest Fisheries Development: A Guide to Handling, Preservation, Processing and Quality. Charthan Maritime, Kent ME4 4TB, United Kingdom. Bergey's Manual of Determinative Bacteriology. 6th Edition 1948. The Williams and Wilkins Co., Baltimore.
27. Yagoub SO. 2009. Isolation of Enterobacteria and *Pseudomonas* species from raw fish sold in fish market in Khartoum State. *Journal of Bacteriological Research* 1(7): 85-88.
28. Yagoub SO, Ahmed TM. 2004. Pathogenic micro-organisms in fresh water samples collected from Khartoum Central Market. *Sudan Jr. Vet. Sci. Animal Husbandry* 43(1/2): 32-37.