## THE BACTERIAL FLORA OF FRESHWATER FISHES FROM FOUR MARKETS OF BHOPAL

### Sumer Hassan, T.A.Qureshi

Dept. of Zoology & Applied Aquaculture, Barkatullah University Bhopal-462026(M.P)

*Abstract:* Bacterial flora of freshwater fishes from four fish markets of Bhopal was accessed. The skin, gills, muscles and intestine of Channa punctatus, Channa striatus, Clarias batrachus, Xenentodon cancila, Mystus cavasius, Labeo rohita, Labeo gonius, Hilsa toli, Chanda nama and Puntius sarana were examined. As a result of investigation five different species of bacteria namely Aeromonas hydrophila, Pseudomonas fluorescence, Salmonella, Shigella, Streptococcus and Staphylococcus were isolated and identified, the public health and economic significance were discussed.

Keywords: Micro-organisms, freshwater fishes, public health, economic significance.

#### I. INTRODUCTION

Water is a natural habitat for a wide variety of micro-organisms including bacteria, protozoa, algae and fungi. Hence, fishes and other aquatic animals may accumulate these organisms from their environment. Fishes from polluted waters may also carry bacteria, derived from human and animal sources. Shewan (1977) indicated that the microbial flora of fish is a function of the environment in which they are caught. While these micro-organisms may not be pathogenic to fish, the role fishes in the possible transfer of pathogens between livestock and humans is important, particularly in less developed countries FAO (2003).Pathogens can affect human health through active and passive contact. fish are involved in both active and passive transfer of a range of parasites and diseases to humans, broadening the need for risk assessment. Threats to public health from aquaculture is diverse as well as the negative impact on the marketability, trade and consumer confidence (Subasinghe et al., 2001).

Over the past three decades aquaculture has expanded, intensified and diversified, based heavily on movements of live aquatic animals and animal products. The rapid expansion of aquaculture continuously surpasses the rate of education, research and adaptation of expertise in health management. Advances in live aquatic animal trade, facilitated by improved transportation efficiency are now recognized as having played a vital role in the introduction and spread of pathogens into many aquaculture systems (Berth, 2000; Humphrey, 2001; Subasinghe and Arthur, 2001).

#### **II. MATERIAL AND METHODS**

Fish samples were collected from four major fish markets of Bhopal viz. Itwara, Bittan, Piplani and Govindpura and transferred to laboratory. Species of fishes include, Channa punctatus, Xenentodon cancila, Mystus cavacius, Labeo rohita, Labeo gonius, Channa striatus, Hilsa toli, Chanda nama and Puntius sarana. The fish smears were obtained from the skin, gills, muscles and intestine.

*Preparation of Smears:* Smears from the skin were obtained from the lateral sides of the body and caudal fin by sterile spatula. Smears from the gills were obtained by clipping the operculum and thereby exposing the gills underneath. Samples from viscera were obtained by opening the body cavity.

# Issue 3, pp: (16-21), Month: July 2014 - September 2014, Available at: www.researchpublish.com www.researchpublish.com

*Microbial Analysis Of Samples:* Inocula was obtained from the smear and inoculated with sterile inoculating loop on Nutrient agar, Pseudomonas isolation agar, Trypticase soy agar, Triple sugar iron agar, Bismuth sulphite agar and Deoxychocolate agar incubated at 37 °C for 24hrs. Colonies were examined, Gram stained and subjected to morphological and biochemical characterizations and identified.

#### **III. RESULTS**

Five Gram negative bacterial species namely, Aeromonas hydrophila, Pseudomonas fluorescence, Salmonella, Shigella, Streptococcus and Staphylococcus were isolated.

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$3.0 \times 10^3$	5.5x10 <sup>3</sup>	$1.5 \times 10^{3}$	$9.0  ext{ x10}^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	$10.5 \times 10^3$	Nil	$1.5 \times 10^{3}$	$5.4 \text{x} 10^3$
fluorescens	CFU/g		CFU/g	CFU/g
Streptococcus iniae	Nil	Nil	Nil	$10.0 \text{x} 10^3$
				CFU/g
Staphylococcus	$2.6 \times 10^3$	$3.5 \times 10^3$	Nil	Nil
aureus	CFU/g	CFU/g		
Salmonella sp.	$1.5 \times 10^{3}$	Nil	$2.5 \times 10^3$	Nil
	CFU/g		CFU/g	

#### Channa puctatus:-

#### Hilsa toli:-

Bacteria	Skin	Gills	Muscles	Intestine
Pseudomonas	$10x10^{3}$	Nil	Nil	$3.5 \times 10^3$
fluorescens	CFU/g			CFU/g
Streptococcus iniae	$4.1 \times 10^3$	Nil	Nil	$3.6 \times 10^3$
	CFU/g			CFU/g
Staphylococcus	$10.0 \text{x} 10^3$	$10.0 \text{x} 10^3$	$5.0 \times 10^3$	$8.4 \times 10^3$
aureus	CFU/g	CFU/g	CFU/g	CFU/g
Shigella sp.	$9.0 \times 10^3$	Nil	Nil	$4.5 \times 10^3$
	CFU/g			CFU/g
Salmonella sp.	$4.4 \text{x} 10^3$	Nil	Nil	$2.5 \times 10^3$
	CFU/g			CFU/g

#### Xenentodon cancila:-

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$3.0 \times 10^3$	$6.5 \times 10^3$	$1.5 \times 10^{3}$	$7.0 \times 10^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	$1.25 \times 10^3$	$1.5 \times 10^3$	$2.5 \times 10^3$	$3.4 \times 10^3$
fluorescens	CFU/g	CFU/g	CFU/g	CFU/g
Streptococcus iniae	$9.1 \times 10^3$	Nil	Nil	9.7x10 <sup>3</sup>
	CFU/g			CFU/g
Staphylococcus	$5.5 \times 10^3$	$3.5 \times 10^3$	Nil	$3.5 \times 10^3$
aureus	CFU/g	CFU/g		CFU/g
Shigella sp.	Nil	Nil	$4.5 \times 10^3$	$10.0 \text{x} 10^3$
			CFU/g	CFU/g
Salmonella sp.	2.5x10 <sup>3</sup> CFU/g	$10.0 \text{x} 10^3$	Nil	$10.5 \text{x} 10^3$
		CFU/g		CFU/g

Bacteria	Skin	Gills	Muscles	Intestine
Pseudomonas fluorescens	2.0x10 <sup>3</sup> CFU/g	Nil	Nil	4.5x10 <sup>3</sup> CFU/g
Streptococcus iniae	6.1x10 <sup>3</sup> CFU/g	Nil	Nil	3.0x10 <sup>3</sup> CFU/g
Staphylococcus aureus	3.6x10 <sup>3</sup> CFU/g	8.0x10 <sup>3</sup> CFU/g	2.0x10 <sup>3</sup> CFU/g	4.4x10 <sup>3</sup> CFU/g
Shigella sp.	7.9x10 <sup>3</sup> CFU/g	Nil	Nil	4.5x10 <sup>3</sup> CFU/g
Salmonella sp.	10.0x10 <sup>3</sup> CFU/g	Nil	Nil	10.0x10 <sup>3</sup> CFU/g

#### Mystius cavacius:-

#### Labeo rohita:-

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$4.0 \text{ x} 10^3$	$5.5 \text{ x} 10^3$	$2.5 \text{ x} 10^3$	$6.0 \text{ x} 10^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	3.1x10 <sup>3</sup>	$0.5 x 10^3$	$1.5 \times 10^{3}$	$2.0 \text{x} 10^3$
fluorescens	CFU/g	CFU/g	CFU/g	CFU/g
Streptococcus iniae	9.1x10 <sup>3</sup>	Nil	Nil	$1.9 \text{x} 10^3$
	CFU/g			CFU/g
Staphylococcus	5.5x10 <sup>3</sup>	$3.5 \times 10^3$	$2.0 \mathrm{x} 10^3$	$3.5 \times 10^3$
aureus	CFU/g	CFU/g	CFU/g	CFU/g
Shigella sp.	$0.6 \times 10^3$	Nil	Nil	$0.41 \times 10^3$
	CFU/g			Cfu/g
Salmonella sp.	$2.5 \times 10^3$	$10.0 \text{x} 10^3$	Nil	$7.5 \text{x} 10^3$
	CFU/g	CFU/g		CFU/g

#### Chanda nama:-

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$4.0 \text{ x} 10^3$	$6.8 \text{ x} 10^3$	$1.5 \text{ x} 10^3$	$6.0  ext{ x10}^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	10.5x10 <sup>3</sup>	10.5x10 <sup>3</sup>	3.5x10 <sup>3</sup>	10.0x10 <sup>3</sup>
fluorescens	CFU/g	CFU/g	CFU/g	CFU/g
Streptococcus iniae	2.9x10 <sup>3</sup>	Nil	Nil	2.55x10 <sup>3</sup>
	CFU/g			CFU/g
Staphylococcus	$1.5 \times 10^3$	$0.5 \times 10^3$	$1.0 \times 10^{3}$	$1.5 \times 10^3$
aureus	CFU/g	CFU/g	CFU/g	CFU/g
Salmonella sp.	$1.5 \times 10^3$	$4.0 \times 10^3$	Nil	6.5x10 <sup>3</sup>
	CFU/g	CFU/g		CFU/g

#### ISSN 2348-313X (Print)

International Journal of Life Sciences Research ISSN 2348-3148 (online)

Vol. 2, Issue 3, pp: (16-21), Month: July 2014 - September 2014, Available at: www.researchpublish.com

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$8.0  ext{ x10}^3$	$6.5  ext{ x10}^3$	$2.5 \text{ x} 10^3$	$4.0 \text{ x} 10^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	$6.5 \times 10^3$	Nil	Nil	$4.25 \times 10^3$
fluorescens	CFU/g			CFU/g
Streptococcus iniae	$10.5 \text{x} 10^3$	Nil	Nil	$9.5 \times 10^3$
	CFU/g			CFU/g
Staphylococcus	$4.0 \mathrm{x} 10^3$	$5.0 \times 10^3$	Nil	$2.4 \times 10^3$
aureus	CFU/g	CFU/g		CFU/g
Shigella sp.	$5.5 \times 10^3$	Nil	$3.0 \times 10^3$	$3.8 \times 10^3$
	CFU/g		CFU/g	CFU/g
Salmonella sp.	$4.2 \times 10^3$	Nil	$4.0  ext{x} 10^3$	$1.5 \times 10^3$
	CFU/g		CFU/g	CFU/g

#### Punctius sarana:-

#### Labeo gonius:-

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas	$2.0 \text{ x} 10^3$	$4.5  ext{ x10}^3$	$1.0 \text{ x} 10^3$	$2.0 \text{ x} 10^3$
hydrophila	CFU/g	CFU/g	CFU/g	CFU/g
Pseudomonas	$6.5 \times 10^3$	Nil	Nil	$4.5 \times 10^3$
fluorescens	CFU/g			CFU/g
Streptococcus iniae	$1.25 \times 10^3$	Nil	Nil	$3.5 \times 10^3$
	CFU/g			CFU/g
Staphylococcus	$1.5 \text{x} 10^3$	$1.0 \mathrm{x} 10^3$	Nil	$6.4 \times 10^3$
aureus	CFU/g	CFU/g		CFU/g
Shigella sp.	$10.0 \text{x} 10^3$	Nil	$4.0 \times 10^3$	$7.0 \times 10^3$
	CFU/g		CFU/g	CFU/g
Salmonella sp.	$4.0 \times 10^3$	Nil	Nil	$5.5 \times 10^3$
	CFU/g			CFU/g

#### **IV. DISCUSSION**

Most of the micro-organisms isolated are found in water and soil and are capable of causing serious diseases in fishes and human beings. The presence of these pathogens is an indication of food-borne illness followed by cramps, abdominal dropsy and gastrointestinal disorders. There is a risk to people who consumes the infested fish especially young children, elderly people, pregnant women and those who handle or prepare fish (FAO, 2003). The fish farmer undergoes economic crunch because of the rejection of the produce and the consumer suffers ill health due to consumption of fish containing zoonotic agents (Babu, 2000).

Health management and personal hygiene practices would ensure good quality, optimize yield and reduce the incidence of food-borne illness.

#### ACKNOWLEDGEMENTS

Authors are thankful to Dept. of Zoology & Applied Aquaculture, Barkatullah University Bhopal-462026(M.P)

#### REFERENCES

 Perez, L., Nunez, F., Rubio, M. and Nicoli, M. (1999). Detection of Salmonella Spp., Shigella (Flexneri and Sonnei) and Vibrio cholerae by PCR in exported shrimp from Mexican northeast coast. Veterinaria Mexico, pp.83-95.

- [2] Phillips, C. A. (1996). Modified atmosphere packaging and its effect on the microbiological quality and safety of produce. International J. food Sci. and Technology,31,463-479.
- [3] Plumb, J. A. (1994). Health maintenance of cultured fishes: Principal microbial diseases. CRC Publication, 1-239.
- [4] Plumb, J. A., Schachte, J. H., Gaines, J. L., Peltier, W. and Carrol, B. (1974). Streptococcus sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. Trans. Am. Fish. Soc., 103:358–361.
- [5] Schmidt, A.M., Schmidt, G. and Sinell, H. J. (1997). Occurrence of Listeria monocytogenes in fish retail stores. Arch. Lebenmittelhyg, 48:105-107.
- [6] Schmidt, K. (1998). Situation of food-borne diseases in Europe, 1992-1996, pp. 262-266. In Proceedings of the 4<sup>th</sup> World Congress on Food borne infections and intoxications, 7-12 June, Berlin.
- [7] Sender, S., Bottcher, K., Cetin, Y. and Gros, C. (1999).Carbonic anhydrase in the gills of Seawater and Freshwater-acclimated Flounders Platichthys flesus: Purification, characterization, and immunohistochemical localization. The Journal of Histochemistry and Cytochemistry, Vol. 47(1): 43–50.
- [8] Shenouda, S.Y.K. (1980). Theories of protein denaturation during frozen storage of fish flesh. Adv. Food Res., 26: 275-311.
- [9] Shanthini, F. and Patterson, J. (2003).Fungi in salted and sun dried fishes of Tuticorin, Southeast coast of India. Seafood Safety Proceedings. Central institute of Fisheries Technology, Cochin.p.412-417.
- [10] Shewan, J. M. (1937). The salt curing of herring. in "Report of the director of food investigation for the year 1937." pp 84-89.
- [11] Shewan, J. M. (1945a). Some of the principles involved in the smoke curing of fish. Chem. Ind ., (London) no.13,98-101.
- [12] Shewan, J. M. (1945b). Bacteriology of dehydrated fish. Qualitative and quantitative studies of the drying process. J. Hyg., 44, 193-209.
- [13] Shewan, J. M. (1949a). Some bacteriological aspects of handling and processing and distribution of fish. J. Roy. Sanit. Inst., 69, 394-421.
- [14] Shewan, J. M. (1953a). Some recent progress in the bacteriology of marine fish. Atti. Congri. Intern. Microbiol.,7, pp. 361-365.
- [15] Shewan, J. M. (1955b). The nitrogenous extractives from fresh fish muscles. J. Sci. Food Agri., 4, pp. 565-568.
- [16] Shewan, J. M. and Liston, J. (1956). Objective and subjective assessments of fish quality. Bull. Inst. Intern. Froid., Annexe1.137-147.
- [17] Shewan, J. M., Hobbs, G. and Hodgkiss, W. (1960). A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the Pseudomonadaceae. J. Appl. Bacteriol., 23:379-390.
- [18] Shewan, J. M. (1961). The microbiology of sea-water fish, Fish as food, Vol. 1. pp.487-560. Academic Press Inc., New York.21.
- [19] Shewan, J. M. (1977). The Bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In Proceedings of handling, processing and marketing of tropical fish conference. Tropical Products Institute, London, pp 51-66. Tropical Products Institute, London.
- [20] Shin, J. W. and Huang, Y. H. (2000). The investigation for contamination of parasite and aerobic bacteria in frozen tilapia fillets in Taiwan. Journal of Food and Drug Analysis, 8 (1): 51-56.
- [21] Siddappaji, S. and Bhandary, M. H. (2003). Spoilage microorganisms in fish sausage. Seafood Safety Proceedings. Central institute of Fisheries Technology, Cochin. pp.442-446.
- [22] Simidu, U. and Hasuo, K. (1968). Salt dependency of the bacterial flora of marine fish. J. gen. Microbiol., 52,355.
- [23] Smith, J. S. and Hui, Y. H. (2004). Food processing: Principles and applications. Blackwell, U.S.A.
- [24] Snieszko, S. F., Bullock, G. L., Dunbar, C. E. and Pettijohn, L L. (1964). Nocardial infection in hatchery reared fingerling rainbow trout (Salmo gairdneri). J.Bacteriol., Vol.88, pp. 1808-1810.
- [25] Snieszko, S. F. (1958). Natural resistance and susceptibility to infections. Progressive Fish Culturist, 20,127-306.

[26] Sonnenwirth, A. C. (1980a). The enteric bacillus and bacteriodes. In: Microbiology 3<sup>rd</sup> ed. (B. D. Davis, R. Dulbecca, H. N. Eisen and H. S. Ginsberg, eds.), pp.668, New York, Harper and Row Publishers Inc.

- [27] Sonnenwirth, A. C. (1980 b). Pseudomonas and other non fermenting bacilli. In: Microbiology 3<sup>rd</sup> ed. (B. D. Davis, R. Dulbecca, H. N. Eisen and H. S. Ginsberg, eds.), pp.674, New York, Harper and Row Publishers Inc.
- [28] Spencer, R. (1956). Flora of cod and the effect of handling. Gt. Brit. Dept. Sci. Ind. Research Rept. Food Invest. Board pp.20-21.
- [29] Spencer, R. (1957). Unpublished data. Humber Laboratory ,Hull, England.
- [30] Spencer, R. (1959). The sanitation of fish boxes, quantitative and qualitative bacteriology of commercial wooden fish boxes. J. Appl. Bacteriol., 22, 73-84.
- [31] Stohr, V., Joffraud, J. J., Cardinal, M. and Leroi, F. (2001). Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. Food Research International, 34 (9) 797 806
- [32] Surendran, P. K., Thampuran, N., Gopakumar, K. (1994): Microbiological profile of cultured fishes and prawn viz a viz their spoilage and contamination. FAO Fisheries Report 514, 1-12.