European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 11, 2020

BACTERIAL DIVERSITY ASSOCIATED WITH *LABEO ROHITA* ISOLATED FROM CULTURED AND NATURAL WATER BODIES

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ABSTRACT

One of the most common carp in all of India is Rohu (Labeo rohita). In both wild and cultivated fish, bacteria are an important causative agent of fish diseases and cause severe losses. Some pathogens, particularly pseudomonads, aeromonads, vibrios etc., are present as skin infections. Human infections caused by these bacterial pathogens are common and associated with the different factors of fish and humans transmitted through fish or water environments.The aim of this work was to identify and isolate certain cases of fish diseases in causative bacterial pathogen with a view to establishing a local database on fish diseases in order to help identify diseases and increase understanding of the fish diseases in Marathwada fish farms.

The Diversity of Bacteria associated with different parts of body namely skin, gills, mouth and intestine of fishLabeo rohita were studied. The different 13 types of bacteria isolated from different parts of body. The predominant bacterial genera associated with this fish were Pseudomonas, Aeromonas, Klebsiella, Proteus, Serratia, Micrococcus, Salmonella, E. coli, but Citrobacter, Staphylococcus, Flavobacterium, Shigella, Bacillus, Enterobacter, Alcaligens, Streptococcus, Corynebacterium, Chromobacterium, Clostridium.

INTRODUCTION

India is one of the world's leading producers of fish with Indian large carp. The most favored animals are catla, rohu, mrigal and pangas.The sustained production of cultured fish leads to a sufficient availability of food to human use. However, the production of fish from fish farming is strongly influenced by fish diseases as compared to cultured fisheries. Fish culture has been severely affected by outbreaks of fish disease. Investigations have been carried out to understand and reduce occurrence of fish diseases in farming. The most frequent cause of fish diseases is bacterial infection in Marathwada.

Snieszko (1958) stated that all animals (including fishes) may be susceptible to some infectious diseases temporarily in their lives. He also suggested that fishes possess some levels of natural resistance to diseases. Nonetheless, natural resistance can be affected by environmental stress and aqua-cultural management practices. According to the host-pathogen-environment theory, in addition to pathogen, unfavourable environmental conditions are required to trigger the development of disease (Wobeser, 2007). Different variables in these factors could affect the disease potential. In normal conditions, healthy fishes can co-exist with pathogens in the environment as their immune system can protect them from infection of the pathogens. However, if there are unfavourable environmental conditions, such as decreased dissolved oxygen concentration, sudden changes in pH, salinities or water temperature, diseases are more likely to occur. When the deterioration of environmental conditions exceeds the host's tolerance limit, stress acting on the host would increase (Snieszko, 1973). The amount of antibodies and the production of antibodies by

circulating lymphocytes in fish would be diminished by stress (Ellis, 1981). As a result, stress would increase the susceptibility to disease in fish (Pickering, 1987; Snieszko, 1974).

One of the most common carp in all of India is Rohu (*Labeo rohita*). In both wild and cultivated fish, bacteria are an important causative agent of fish diseases and cause severe losses. Some pathogens, particularly pseudomonads, aeromonads, vibrios etc., are present as skin infections. Human infections caused by these bacterial pathogens are common and associated with the different factors of fish and humans transmitted through fish or water environments. Such pathogens are also pathogenic to both fish and humans as the bacteria are facultative parasitic bacteria. It may be distinguished from fish with or without apparent disease signs.

In Marathwada no systematic research on bacterial disease in fish has been carried out. Therefore, the study of aquatic bacteria associated with fish is very limited in Marathwada region of Maharashtra. Our earlier work was a sincere attempt to assess the bacterial population in aquatic environment and their involvement in causing diseases in fish. Darak and Barde (2015) reported *Aeromonas* sp. and *Pseudomonas* sp. are very common bacteria associated with major carp and live fishes. The study was to identify the common bacterial pathogen associated with freshwater fish *Labeo rohita*and characterize the pathogen in detail with their susceptibility to antibiotics for developing a control measure in future.

The aim of this work is to identify and isolate certain cases of *Labeo rohita* diseases in causative bacterial pathogen with a view to establishing a local database on fish diseases in order to help identify diseases and increase understanding of the fish diseases in Marathwada fish farms.

MATERIAL AND METHOD

Study area

The study was carried out in two systems, 1) A natural riverine system like godavari and other rivers with considerable population of fishes used in the study. 2) An artificial fish cultivation system where water bodies were used for culturing the fishes used in the study.

Sr.No	Location / District	Natural system	Cultured system
1	**Nanded**	Godavari (Vishnupuri)	Kandhar
		Asna (Devapur)	Petwadaj
2	**Hingoli**	Kayadu (Balapur)	Bhategaon
3	**Parbhani**	Masoli (Gangakhed)	Yeldari
4	**Aurangabad**	Nagzhari (Tembapuri)	Paithan
5	**Jalna**	Galhati (Ambad)	Ghanewadi
6	**Latur**	Manjra (Latur)	Sakol, Udgir
7	**Beed**	Sindphana (patoda)	Mehakari, Ashti
8	**Osmanabad**	Sina (Paranda)	Khasapur

Collection of fishes

Samples were obtained at monthly intervals for water and fish for bacteriological studies. Aseptically collection of water samples from the surface and approximately 100 cm below the surface of the water in sterilized screw capped bottles was done. In order to reduce contamination by storage, bottles were pre-wrapped with filter paper. Samples were taken for

analysis after processing at the laboratory immediately. Immediately after catching with sterilized cotton swabs, samples of different body parts were obtained. If no immediate analysis is necessary, the samples were stored at 4 °C until 6 hours. Within 4 hours of selection the samples were cultivated.

*Labeo rohita*have been collected typically from surrounding natural bodies of water and cultivated systems. After determining the source, some fish were also bought from the local markets. The fishes were selected and considering their easier availability, the health status of the fishes was given high priority.

Maintenance of fishes

The experimental fishes were maintained in the laboratory in a glass aquarium of 100 x 50 x 50 cm with static water depth was between 25 and 30 cm. The temperature of water was maintained held at 28±2 °C. The fish are fed with cut earthworms regularly. There were 20 fishes in one tank in a weight range from 20 to 30 gms. Higher weight fish were not considered for experiment because of handling difficulties. All fish were acclimatized in laboratory conditions at least ten days before use in experimental studies. The collection of fishes was discarded, if significant fish were dead within 10 days of acclimatization.

Bacterial cultures

For the bacterial diversity and bacterial load, the bacterial parameters of the widely cultivated and consumed fish have been evaluated and compared for both cultivated and natural systems. The fishes were retrieved with the help of fishing net whereas the samples of water were collected in sterile bottles and polythene bags.

Isolation, identification and Enumeration of bacteria

The isolation of heterotrophic, aerobic and anaerobic bacterial communities in terms of cfu / ml were identified and enumerated by the standard methods described by Cheesbrough (1989) and Bergey's Manual for Systematic Bacteriology (1986).

RESULT AND DISCUSION

Bacterial diversity of various body parts of *Labeo rohita* from cultured system

The bacterial diversity of various body parts of *L. rohita* were represented in Table 1.

The predominant bacteria obtained from the skin of *Labeo rohita* were *Klebsiella, Aeromonas, Pseudomonas, E. coli, Clostridium, Proteus, Chromobacterium, Bacillus, Staphylococcus, Citrobacter, Micrococcus* but *Salmonella, Corynebacterium, Shigella, Enterobacter, Streptococcus, Flavobacterium, Alcaligens, Serratia* were found to be moderate.

The predominant bacteria obtained from the mouth cavity of *L.rohita* were *Salmonella, Aeromonas, Chromobacterium, Pseudomonas, Bacillus, Citrobacter, Clostridium, Proteus,* but *Flavobacterium, Enterobacter, Klebsiella, E. coli, Serratia, Staphylococcus, Alcaligens, Corynebacterium, Shigella, Streptococcus, Micrococcus* were found to be moderate.

The predominant bacteria found in the gills of *L. rohita* included *Aeromonas, Alcaligens, Pseudomonas, Serratia, Corynebacterium, Shigella, Bacillus, Staphylococcus,*

Flavobacterium but *Clostridium, Enterobacter, E. coli, Micrococcus, Chromobacterium, Citrobacter, Proteus, Klebsiella, Streptococcus*were found to be moderate.
The predominant bacteria found in the intestine of *L. rohita* included *Aeromonas, Alcaligens, Citrobacter, Pseudomonas, Enterobacter, E. coli, Salmonella, Bacillus, Streptococcus, Clostridium,* but *Klebsiella, Serratia, Proteus, Staphylococcus, Chromobacterium, Flavobacterium, Micrococcus, Corynebacterium, Shigella*were found to be moderate.
The predominant bacteria isolated from the various body parts of *L.rohita* showed the predominance of *Klebsiella, Aeromonas, Pseudomonas, E. coli*..
Since then, several reports were published on the bacterial diseases of fish, in both fresh and salt water, and ample literature is available in this speciality. Several books on fish diseases and useful Schaperclaus (1986) , reviews had also been published by Davis (1953), Reichenbach-Klinke (1977), Dulin (1966), Sindermann (1977) Bullock et al. (1971), Goldstein (1971), Mawdesley-Thomas(1972) , Roberts and Shepherd (1974) and Schubert (1974).

Table 1 Bacterial diversity of various body parts of *L. rohita* from cultured system

Bacterial genera	Number of bacteria (CFU/ml) $X10^2$			
	skin	Mouth	Gills	Intestine
Aeromonas	7.63	5.16	4.75	4.05
Alcaligens	1.24	1.44	4.12	3.94
Bacillus	5.74	4.46	2.85	2.16
Chromobacterium	6.45	4.85	1.29	1.45
Citrobacter	4.54	4.35	1.12	3.75
Clostridium	6.75	4.16	1.75	2.05
Corynebacterium	3.14	1.14	3.36	1.35
E. coli	6.94	2.33	1.63	3.25
Enterobacter	2.38	3.28	1.75	3.45
Flavobacterium	1.33	3.41	2.03	1.44
Klebsiella	8.24	2.41	1.02	1.93
Micrococcus	4.43	1.02	1.35	1.44
Proteus	6.64	4.03	1.03	1.64
Pseudomonas	7.24	4.78	4.02	3.63
Salmonella	3.74	5.46	2.85	2.16
Serratia	1.24	1.63	3.45	1.69
Shigella	3.14	1.14	3.36	1.35
Staphylococcus	5.54	1.45	2.50	1.59
Streptococcus	1.84	1.08	1.02	2.16

Bacterial diversity of various body parts of *Labeo rohita* from Natural system
The bacterial diversity of various body parts of *L. rohita* were represented in Table 2.
The predominant bacteria obtained from the skin of *Labeo rohita* were *Pseudomonas, Proteus, Aeromonas, E. coli, Clostridium, Salmonella, Staphylococcus, Chromobacterium,*

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Klebsiella, Bacillus, Citrobacter, Micrococcus, Corynebacterium, but *Shigella, Enterobacter, Streptococcus, Flavobacterium, Alcaligens, Serratia* were found to be moderate.

The predominant bacteria obtained from the mouth cavity of *L.rohita* were *Pseudomonas, Salmonella, Aeromonas, Chromobacterium, Bacillus, Citrobacter, Clostridium, Proteus, Flavobacterium, Enterobacter,* but *Klebsiella, E. coli, Serratia, Staphylococcus, Alcaligens, Corynebacterium, Shigella, Streptococcus, Micrococcus*were found to be moderate.

The predominant bacteria found in the gills of *L. rohita* included *Pseudomonas, Aeromonas, Serratia, Corynebacterium, Shigella, Alcaligens,* but *Salmonella, Bacillus, Staphylococcus, Flavobacterium, Clostridium, Enterobacter, E. coli, Micrococcus, Chromobacterium, Citrobacter, Proteus, Klebsiella, Streptococcus* were found to be moderate.

The predominant bacteria found in the intestine of *L. rohita* included *Pseudomonas, Aeromonas, Alcaligens, Citrobacter, Enterobacter, E. coli, Salmonella, Bacillus, Streptococcus, Clostridium,* but *Klebsiella, Serratia, Proteus, Staphylococcus, Chromobacterium, Flavobacterium, Micrococcus, Corynebacterium, Shigella*were found to be moderate.

The predominant bacteria isolated from the various body parts of *L.rohita* showed the predominance of *Pseudomonas, Salmonella, Aeromonas, Chromobacterium, Bacillus,*.Prior to the recent outbreak of epizootic ulcerative syndrome in India, several workers had reported ulcerative fish diseases occurring in Indian waters affecting mostly the Indian major carps. Gopalkrishnan (1964) reported many instances of *Aeromonas hydrophila* infections among carps in the state of West Bengal. Pal *et* al (1978) reported skin lesions in *Anabas testudineus.* Incidence of opercular ulcer disease in *Labeo rohita* at a private carp culture tank in the state of Tripura was reported by Lipton (1983). Other workers (Manohar *et al,* 1976; Pal, 1984) also reported occurrence of dermal ulcers in Indian major carps and economically important catfishes. An ulcerative form of *Aeromonas hydrophila* infection was investigated by Karunasagar *et* al (1986) which occurred in the state of Andhra Pradesh. Though on all such occasions *A. hydrophila* was isolated from the lesions, various bacteria were associated as secondary pathogens. In spite of several attempts, no bacteria could be isolated from the internal organs. However, there was no extensive spread of the disease and the disease condition was effectively controlled through chemical treatment. *Labeo rohita*had been found to be the most susceptible species to the disease. Bilateral ulcerations of the opercula and the head and sometimes deep ulcers penetrating the cranial and opercular bones were observed (Kumar et al., 1991).

Table 2: Bacterial diversity of various body parts of *L. rohita* from Natural system

Bacterial genera	Number of bacteria (CFU/ml) $X10^2$			
	skin	Mouth	Gills	Intestine
Aeromonas	6.63	5.16	4.75	4.05
Alcaligens	1.24	1.44	3.12	3.94
Bacillus	4.74	4.46	2.85	2.16
Chromobacterium	5.45	4.8	1.29	1.45
Citrobacter	4.54		12	3.75

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Clostridium	5.75	4.16	1.75	2.05
Corynebacterium	3.14	1.14	3.36	1.35
E. coli	5.94	2.33	1.63	3.25
Enterobacter	2.38	3.28	1.75	3.45
Flavobacterium	1.33	3.41	2.03	1.44
Klebsiella	5.24	2.41	1.02	1.93
Micrococcus	4.43	1.02	1.35	1.44
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Shigella	3.14	1.14	3.36	1.35
Staphylococcus	5.54	1.45	2.50	1.59
Streptococcus	1.84	1.08	1.02	2.16

Since the first appearance of the epizootic ulcerative syndrome in India, it was distinct by its destructive nature and capacity of affecting a wide variety of fish species in both wild and cultured waters. Conditions became so alarming that within two years of its outbreak all fishing activities came to a standstill causing tremendous concern to the fishery scientists and administrators. The disease spread alarmingly and it was accepted that no fish disease in India has been as virulent and menacing as the recent outbreaks of the epizootic ulcerative syndrome (Das et al, 2007).

ACKNOWLEDGEMENT:

This research work was financially supported by Minor Research Project Grant of Swami Ramanand Teerth Marathwada University, Nanded vide letter no:APDS/Uni.MRP/ Sci.& Tech.:Zoology. / 2019-20/2838 dt.13.01.2020

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