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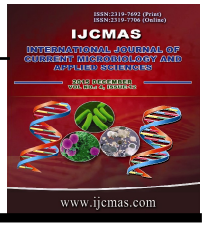

Original Research Article

Identification of Enterobacteriaceae studies in Carps during rearing a fresh water pond

R. Saraswathi[1], P. Sumithra[1] and R. Sivakami[2]*

[1]Department of Microbiology, Srimad Andavan Arts & Science College, Tiruchirappalli-620 005, Tamil Nadu, India
[2]Department of Zoology, Arignar Anna Govt. Arts College, Musiri-621 211, Tamil Nadu, India
**Corresponding author*

Keywords

Entero-bacteriaceae, Cultured carps, Public health

A B S T R A C T

Until now, microbiological studies on cultured freshwater fish appear to be limited especially in India. Hence, a study was done to identify the bacterial populations and pathogens in pond water and in some tissues of cultured carps *Catla catla, Labeo rohita, Cirrhinus mrigala and Cyprinus carpio* polycultured in a freshwater pond (Koppu temple pond) to evaluate the public health aspects associated with the consumption of these fishes. Among the various organs analyzed, the maximum bacterial load was found in skin followed by gills and intestine for all the fishes. The enteric bacteria isolated from the freshly caught fish were found to belong to 10 genera involving a total of 13 species. The bacterial species isolated from water also belonged to the same 10 genera involving a total of 14 species. In general, the bacteria isolated from fish samples appeared to be very similar to those obtained from water. Among the various bacteria present in the fishes, *E. coli* and *P. aeruginosa* contributed the most (*L. rohita* - 55.5%; *C. carpio* - 52.06%; *C. mrigala* - 40.18% and *C. catla* - 38.0%) with the values ranging from 38% (Catla) to 55.50 % (Rohu). In the water also, the most dominant bacterial species was again *E. coli* and *P. aeruginosa*. Thus, there appears to be a close correlation between the bacteria present in water and in organs of fishes with E. coli dominating followed by *P. aeruginosa* in all the four types of fishes. The presence of a large number of enteric bacteria including pathogenic species in the pond suggests the need for following strict hygienic methods during the process of handling and processing of fish in these systems. Besides, it also highlights the need for proper method before consumption of these fishes to prevent humans from getting diseases.

Introduction

India has witnessed an overwhelming growth in the aquaculture sector for the past two decades. It is presently ranked second in aquaculture production. Among the various culture fisheries, carp culture plays an indispensable role in fresh water aquaculture production of India (FAO, 2005). While this growth is much appreciated in terms of food security, the health risks associated with the aquaculture produce is of important concern.

Only recently, has attention been given to microbiological studies focusing on bacterial isolation aimed at establishing causes of fish diseases and medication methods. Further only minimal attention has been given to the composition of the microflora, its variations in time and effect on fish (Zmyslowska *et al.,* 2001).

In recent times, increased attention is given to the possibility of cultured fish as vector of human pathogenic bacteria (Apun *et al.,* 1999; Islam *et al.,* 2000). Fish living in natural environment are known to harbour pathogenic Enterobacteriaceae (Pillay, 1990). Invasion of fish muscles due to the breakage of immunological barrier by pathogens is likely to occur when the fish are raised in ponds containing high levels of faecal coliforms, *E. coli* and *Salmonella* sp. (Guzman *et al.,* 2004). Ogbondeminu and Okoeme (1989) reported that 50% of the microorganisms recovered from both fish and water of earthern pond fertilized with animal faecal waste were members of the family Enterobacteriaceae. In addition, most diseases in humans are caused by opportunistic enteric pathogens, which are prevalent in the rearing environments (Brock, 1993; Jayasree *et al.,* 1999). Reports of the occurrence of pathogenic strains of *E. coli* from fisheries sources and outbreaks of illness due to them are also increasing (Samadpour *et al.,* 1994; Kumar *et al.,* 2001; TeoPhilo *et al.,* 2002; Surendraraj *et al.,* 2009; Sivakami *et al.,* 2011).

Hence, increased attention is given to the possibility of fish acting as vectors of human pathogenic bacteria (Hejkal *et al.,* 1983; Rice *et al.,* 1987; Buras *et al.,* 1987) as a wide range of bacteria like *Escherichia coli*, *Mycobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Salmonella* sp., *Shigella* sp., *Proteus* sp., and *Enterococcus* species have been isolated from skin, digestive tracts, kidney and muscle of different fish species of both temperate and tropical waters (Sakata *et al.,* 1980; Ogbendeminu, 1993). Until now, microbiological studies on cultured freshwater fish appear to be limited especially in India (Surendran and Gopakumar, 1991; Sivakami *et al.,* 1996; 2011; Surendraraj *et al.,* 2009). Hence, the object of the present study was to identify the bacterial populations and pathogens in pond water and in some tissues of cultured carps *Catla catla, Labeo rohita, Cirrhinus mrigala* and *Cyprinus carpio* polycultured in a freshwater pond (Koppu Temple Pond) to evaluate the public health aspects associated with the consumption of these fishes. The information obtained would allow a better control on handling and processing of fishes in these systems by reducing the microbial health hazards.

Materials and Methods

The pond from which fish were sampled was the Koppu temple pond, Tiruchirappalli, Tamil Nadu, India where polyculture of carps was practiced.

Water samples for microbiological analyses were collected, put aspetically into sterile 500 ml sampling bottles and examined within 1-2 hours of collection in the laboratory. All water samples were analysed for the presence of total and faecal coliform bacteria, faecal Streptococci and pathogenic *Salmonella* by the most probable number (MPW) method following the American Public Health Association (APHA 1980) 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 muscle, gills and digestive tracts 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 0.1 ml 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 faecal coliform (M-FC) agar were inoculated in duplicate and incubated at 37°C for 24 hrs. After distinct coloured colonies of various bacteria developed on the plates, further identification of the bacterial colonies were done according to Edwards and Ewing (1972), Cowan (1974), Martin and Washington (1980), Brenner (1984) and Cheesbrough (1989).

Results and Discussion

Table-1 records the various physicochemical variables analysed in water during the sampling day. Tables-2 and 3 present the size and weight ranges of the various carps used for the present study. All the fishes were almost of the same size and weight except for *Labeo rohita* which was longer in size. Tables 4 and 5 record the bacterial counts in water and among the various carp tissues. A comparison of the bacterial count in the skin among the four carps reveals that *Cyprinus carpio* recorded the maximum count followed by *Cirrhinus mrigala*, *Labeo rohita* and *Catla catla*. Amongst the gills, the maximum load was found in *C. catla* followed by *C. mrigala*, *L. rohita* and *C. carpio*. However, bacterial counts in intestine reveals that the maximum load was found in *C. mrigala* followed by *C. catla*, *L. rohita* and *C. carpio*. Thus, in general, among the various organs analyzed, the maximum bacterial load was found in skin followed by gills and intestine for all the fishes.

The enteric bacteria isolated from the freshly caught fish were found to belong to 10 genera involving a total of 13 species. The bacterial species isolated from water also belonged to the same 10 genera involving a total of 14 species. Thus, in general, the bacteria isolated from fish samples appeared to be very similar to those obtained from water. Among the various bacteria present in the fishes , *E. coli* and *P. aeruginosa* contributed the most (*L. rohita* - 55.5%; *C. carpio* - 52.06%; *C. mrigala* - 40.18% and *C. catla* - 38.0%) with the values ranging from 38% (Catla) to 55.50 % (Rohu). In the water also, the most dominant bacterial species was again *E. coli* and *P. aeruginosa*. Thus, there appears to be a close correlation between the bacteria present in water and in organs of fishes with *E. coli* dominating followed by *P. aeruginosa* in all the four types of fishes. A close perusal of the bacteria present in water and fish samples further reveals that in *Catla catla*, *Vibrio parahaemolyticus* was absent while in *Labeo rohita*, *Enterobacter aerogenes* and *Salmonella* sp were absent; in *Cirrhinus mrigala*, *Proteus mirabilis* and *Klebsiella* sp were absent and in *Cyprinus carpio*, *Enterobacter cloacae*, *Proteus rettgeri* and *Salmonella* were absent.

Literature indicates that the intestinal bacterial flora from freshly caught fishes of other ponds also included *Enterobacter* sp, *Proteus* sp., *Salmonella* sp., *Pseudomonas* sp., *Klebsiella* sp., *Serratia* sp., *Shigella* sp. and *Yersinia* sp (Souter *et al.,* 1976; Ogbondeminu, 1993; Sivakami *et al.,* 1996; 2011; Surendraraj *et al.,* 2009). These observations are in line with those of the present study.

Table.1 Physico-Chemical Parameters of Freshwater Pond

S. No.	Parameters	Units	Result
1.	Atmospheric Temperature	°C	27
2.	Water Temperature	°C	26
3.	Colour		Little bit muddy
4.	pH		8.6
5.	Dissolved Oxygen	mg/l	8.97
6.	Carbon dioxide	mg/l	16.79
7.	Total alkalinity	mg/l	245
8.	Phenolphthalein Alkalinity	mg/l	34
9.	Methyl Orange Alkalinity	mg/l	120
10.	Phenolphthalein Alkalinity	mg/l	18
11.	Hardness	mg/l	136
12.	Calcium	mg/l	24.66
13.	Nitrates	mg/l	0.017
14.	Nitrites	mg/l	0.012
15.	Phosphates	mg/l	0.110
16.	Silicate	mg/l	0.092
17.	Chlorides	mg/l	49.6
18.	Total solids	mg/l	945
19.	Magnesium	mg/l	32.2

Table.2 Size and Weight Ranges of the Fish Species used in this Study

S. No.	Name	Size Range (cm)	Average Size (cm)	Weight Range (g)	Average Range (g)
1.	*Catla catla*	34.2-41.6	37.82	530.2-620.7	577.79
2.	*Labeo rohita*	51.7-58.4	54.53	510.6-633.7	584.42
3.	*Cirrhinus mrigala*	36.3-44.1	40.18	580.2-710.6	630.10
4.	*Cyprinus carpio*	37.4-47.9	42.28	600.8-810.4	684.18

Table.3 Bacterial Counts in Pond Water (per millitre)

S. No.	Total Bacterial Count	Room Temperature	Total Bacterial Count at 37 °C	Total Coliforms (MPN)*	*Faecal Streptococci*
1.	2.42×10^{7}	30 °C	8.12×10^{6}	6.42×10^{2}	460

*MPN: Most Problem Number

Table.4 Bacterial Counts in Skin Surface, Gills and Intestine in Four Carp Species (Means of 10 Specimens)

S. No.	Tissues	Fish	Total Bacterial Count at RT	Total Bacterial Count at 37 °C	Total Coliforms	Coagulase Positive *Staphylococci*	*Faecal Streptococci*
1.	Skin surface (Counts g^{-1})	*Catla catla*	2.94×10^{4}	6.98×10^{3}	50	0	48
		Labeo rohita	3.98×10^{3}	9.42×10^{2}	80	32	70
		Cirrhinus mrigala	3.12×10^{4}	10.40×10^{3}	130	26	20
		Cyprinus carpio	2.04×10^{3}	11.42×10^{2}	40	0	40
2.	Gills (Counts g^{-1})	*Catla catla*	3.92×10^{5}	10.98×10^{4}	280	0	180
		Labeo rohita	6.42×10^{4}	5.12×10^{4}	260	10	170
		Cirrhinus mrigala	2.80×10^{5}	11.42×10^{4}	420	0	140
		Cyprinus carpio	2.62×10^{3}	3.42×10^{4}	250	0	120
3.	Intestine with Contents (Counts g^{-1})	*Catla catla*	7.52×10^{4}	10.14×10^{6}	1220	0	260
		Labeo rohita	5.94×10^{6}	4.42×10^{5}	760	0	240
		Cirrhinus mrigala	9.72×10^{5}	8.00×10^{5}	3020	0	300
		Cyprinus carpio	7.72×10^{6}	6.41×10^{5}	1020	0	280

RT - Room Temperature

Table.5 Distribution (%) of Enteric Bacterial Species from Intestinal Tracts of Fish and Pond Water

S. No.	Bacteria	Water	*Catla catla*	*Labeo rohita*	*Cirrhinus mrigala*	*Cyprinus carpio*
1.	*Escherichia coli*	20.78	19.40	35.34	21.42	29.64
2.	*Enterobacter cloacae*	7.43	9.27	7.16	6.44	Nil
3.	*Enterobacter aerogenes*	5.60	5.80	Nil	7.20	10.20
4.	*Proteus rettgeri*	2.89	2.50	3.16	8.40	Nil
5.	*Proteus mirabilis*	5.96	5.20	4.26	Nil	4.26
6.	*Proteus vulgaris*	7.60	6.40	6.20	9.20	8.42
7.	*Salmonella typhi*	4.20	1.10	Nil	10.42	Nil
8.	*Pseudomonas aeruginosa*	14.44	18.60	20.16	18.76	22.42
9.	*Klebsiella pnemoniae*	9.22	4.80	7.42	Nil	4.42
10.	*Aeromonas hydrophila*	3.88	9.93	2.30	2.16	4.64
11.	*Vibrio alginolyticus*	4.02	2.93	3.00	3.10	3.00
12.	*Vibrio parahaemolyticus*	1.02	Nil	0.70	0.60	0.70
13.	*Staphylococcus aureus*	2.02	1.40	1.40	2.30	1.60
14.	*Streptococous faecalis*	4.40	3.00	3.00	4.20	4.40
15.	Unidentified Isolates	6.30	6.00	10.10	6.00	6.20

Previous studies on the bacterial microflora of some fresh water fishes in tropical water (Fasanya *et al.,* 1988; Apun *et al.,* 1999; Islam *et al.,* 2000; Kumar *et al.,* 2001; Teoplulo *et al.,* 2002; Surendraraj *et al.,* 2009; Sivakami *et al.,* 2011) also showed that the most predominant organism isolated from the skin and gills of fishes belonged to the Enterobacteriaceae family as has also been reported in the present study.

The bacterial composition in all the fish species appeared to be a reflection of the bacteria found in the pond water. Several authors (Geldreich and Clarke, 1966; Nieto *et al.,* 1984; Buras *et al.,* 1987; Ogbondeminu, 1993; Surendararaj *et al.,* 2009; Sivakami *et al.,* 1996; 2011) have also reported that the bacterial flora of fish is a reflection of their respective environments.

Thus it is quite natural that *E. coli* and *P. aeruginosa* which are the dominant bacteria in water also dominated in all the fishes cultured. While the presence of *E. coli* can be attributed to the addition of animal manure, the entry of *P. aeruginosa* into the pond might be due to water containing these bacteria from the nearby areas entering into the pond as surface runoff water.

Thus, it appears that it is more likely to be a water related problem then a fish related one because the bacteria appears to enter the fish through the media. Nevertheless, the presence of a large number of enteric bacteria including pathogenic species in the pond suggests the need for following strict hygienic methods during the process of handling and processing of fish in these systems. Besides, it also highlights the need for proper method before consumption of these fishes to prevent ourselves from getting diseases.

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