Enterobacteriaceae group of Organisms in Sewage-Fed Fishes

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Abstract

The skin, gut and muscle of the fish varieties "rohu", (*Labeo rohita*) and the fringe-lipped carp, (*L.fimbriatus*) grown in (I)freshwater,(ii) sewagefed water and (iii)'reclamied' water were examined for the Enterobacteriaceae group of organisms. *Salmonella* sp. did not occur in any of the fishes tested. *Shigella flexneri* was isolated from the fishes reared in sewage fed to freshwater ponds. However, in' reclamied' fishes, *S.flexneri* was present in the gut and skin and not in the muscle tested after 3 weeks of transfer from sewage-fed to freshwater ponds. The steamed, fried and fish curry preparations examined revealed the complete absence of any of the bacteria including the pathogens. The bacteriological quality of the ambient water samples from different sources indicated higher incidence of (i) Total bacteria (ii) Coliform group and (iii) faecal streptococci in the sewage-fed pond.

Introduction

The utilization of human and animal waste to fertilize water for improving the growth of aquatic life has been well documented. In Tamil Nadu Agricultural University, the sewage from the residential colony and the Hostel is lead to an oxidation pond and subsequently diluted in fish culture ponds. Experiments conducted by incorporating a seven species combination (Indian major carps, rohu, mrigal, comman crap; the Chinese carps, silver and grass carps and milk fish) have proved very successful yielding up to 8.5 tones of fish/hectare/year. The present study on the screening of pathogens is conducted on the fish and water samples from this culture unit. There is a much scope for expansion of sewage-fed fish culture in various places like municipalities, townships, hostel etc. where there is an organized sewage disposal system. However, one should approach this problem with appropriate precautions to prevent the diseases that might otherwise be transmitted through such fishes cultured in sewage fed ponds. Hence attempt is made in this study to screen the sewage-fed fishes for the Enterobateriaceae group of organism including the pathogens.

Materials and Methods

Indian major carp, rohu (*Labeo rohita*)and fringe-lipped carp (*L.fimbriatus*) were reared (I) completed in fresh water ponds with artificial feed (Group I) (ii) completed in ponds fed with stabilized domestic sewage without any supplement feed (Group II) and (iii) partly in sewage-fed ponds and partly in fresh water (fish cultered for one year in sewage-fed water without any supplementary feed and subsequently for one month in fresh water with artificial feed) (Group III).

These fishes were screened for the presence of Enterobacteriaceae group of organisms such as *Shigella flexneri*, *S.boydii, S.dyensteriae, S. sonnei, Salmonella* sp. *Escherichia coli, Aeromonas* sp., *Proteus* sp., *Citrobacter* sp., *Klebsiella* sp.

The ambient water samples from fish ponds were collected and examined for coliform and facecal streptococci.

Sensory assessment of the fish, rohu, grown in the groups of ponds was carried out by (1)Steaming for 10 minutes (2)frying with oil and condiments and (3) making curry as per normal kitchen practice and the muscle was for pathogens.

Results and Discussion

The various organisms enumerated from the fishes cultured in different ponds are presented in Table1.While *Shigella flexari* was present in the samples of fishes examined from the sewage-fed ponds (GroupI), is was not encountered in fishes grown in fresh water (GroupII). In 'reclamied' fishes (GroupIII) *S.flexneri* was present in all tissues within a week after transfer of the fish to fresh water, but tests done after 3 weeks revealed the presence of the *Shigella* in the gut and skin and not in the muscle (Table1). *Salmonella* spp did not occur in any of the three groups of fishes. Species other than *S.flexari* namely *S.boydii, S.dysenteriae* and *S.sonnei* were not encounted in the tests while *Escherichia coli* was obtained from the gut specimen of the fish Labeo rohita cultured in group I, II, and group III. The other organisms isolated viz. *Enterobacter* sp: *Klebsiella* sp., *Aerobacter* sp.and non-fermenting rods from the various specimen of the fishes are represented in Table1.

The muscles from the steamed, fired and fish curry were screened for the pathogwens. It was significant to note that of the Enterobacteriaceae group of organisms could be observed in all the samples tested, (Table 2). The thermal-death point of non-sporing, mesophilic bacteria lies between 100-120c for ten minutes (cruickshank,1968). The cooking experiments with exposure

temperature ranging from100-160c for ten mintues in the present study might have resulted in killing all the organisms belonging to the Enterobacteriaceae. As such the transmission of pathogens, if any, in the sewage-fed fishes is unlikely.

The microbial load of the skin, muscle and gut of both the fish varieties examined for (ii) Total bacteria count and (ii) coliform count are depicated in Table3. In general, highest total as well as coliform bacteria were recovered in the gut contents than that of the skin and muscle. Fathima (1973) Mary *et al* (1975) have reported highest bacterial population I the gut of Indian mackeral Rostrelliger kanagurta and three estuarine fishes respectively. It is quite possible that the highest incidence of bacterial population in the gut of these fishes examined may be due to the food ingested and also due to the occurrence of natural flora in the other tissues examined.

The bacteriological quality of different sources of water utilized for fish culture revealed higher (I) total microbial population (ii) coliforms and (iii) faecal streptococci in the sewage-fed ponds (Table4). It is of interest to note that there is a gradual reduction in the population load of bacteria starting from the inlet sewage water to the fish ponds (Table 5). This declining trend might be due to oxidation and dilution of sewage water.

Conclusion

Through significance of fish culture in sewage with a view to maximize production is recognized, successful implementation of such a system still remains to gain momentum. From the point of view of public health, utilization of sewage for fish culture ensures a better way of disposal of this waste water. Further studies may be attempted to make the fishes reared in sewage-fed ponds to running water cisterns and maintained with normal feed for various periods of time. or else, feeding trials with feed mixed with antibiotics and exposure to antibiotic solution may be of value in the control of pathogens, if any, in sewage-fed fishes. Of course, at all times cooking and boiling temperature would eliminate the possibility of any pathogen transmission even if they are contaminated. Apart from providing the much needed

Table I

Enumeration of Enterobacteriaceae Group of Organisms in Rohu (*Labeo rohita*) Fimbriatus (*Labeo fimbriatus*) in different habitat

Organism	Rohu									Fimbriatus								
	Skin			Muscle			Gut			Skin			Muscle			Gut		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Shigella sp.	+	-	+	+	-	-	+	-	+	+	-	+		- -	-	+	-	+
Enterobacter sp.	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-
Klebsiella sp.	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
Aerobacter sp.	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	+	-	-
Non- fermenting rods	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-
Others (Atypical *E.coli* etc)	+	-	+	+	-	-	+	-	+	+	+	-	-	-	-	-	+	+

I : Cultured in Sewage - fed ponds
II : Cultured in Freshwater ponds
III : Cultured in Freshwater ponds for 21 days after growing for one year in sewage - fed ponds

Table II

Screening of fishes grown in sewage-fed ponds for the presence of Enterobacteriacene Group of Organisms

Organism	Uncooked (Fresh samples)						Cooked muscle (Rohu)		
	Labeo rohita			*Labeo fimbriatus*					
	Skin	Muscle	Gut	Skin	Muscle	Gut	Steamed	Fried	Curry
Shigella sp.	+	+	+	+	-	+	-	-	-
Enterobacter sp.	-	+	-	-	+	-	-	-	-
Klebsiella sp.	-	-	+	-	-	-	-	-	-
Aerobacter sp.	-	-	+	-	-	-	-	-	-
E.coli	-	+	+	-	+	+	-	-	-
Non- fermenting rods	-	-	-	-	-	+	-	-	-
Others (Atypical *E.coli* etc)	+	+	+	+	-	-	-	-	-

Table III

Microbial population in the Fish samples cultured in sewage fed and freshwater ponds (Expressed in 10^5 / g of sample)

Fish Variety	Part examined	Sewage-fed pond		Freshwater pond	
		Total Bacterial Count	Coliform Count	Total Bacterial Count	Coliform Count
Rohu	Skin	27.69	16.50	0.93	0.07
	Muscle	36.01	3.40	0.40	0.03
	Gut	100.10	26.69	2.47	0.13
Fimbriatus	Skin	18.87	8.12	0.82	0.04
	Muscle	11.16	3.05	0.29	0.01
	Gut	131.00	15.86	1.93	0.24

Table IV

Bacteriological quality of different sources of water utilized for Fish Culture			
Organism	**Freshwater**	**Sewage water**	**Reclaimed water**
Total bacterial Count (Expressed in 10 6 /ml)	6.62	45.02	8.16
MPN Coliform Count/ 100 ml	$0.22 * 10^5$	$190 * 10^5$	$0.23 * 10^5$
MPN faccal Streptococcal Count/ 100 ml	$11.32 * 10^2$	$213.25 * 10^2$	$10.15 * 10^2$

(Figures represent mean of four values)

Table V

Bacteriological quality of Water samples from Sewage Fish Culture Unit			
Organism	**Sewage inlet**	**Oxidation pond**	**Fish pond**
Total bacterial count/ml	$93.27 * 10^6$	$61.32 * 10^6$	$45.02 * 10^6$
Coliform count/100 ml	$280 * 10^5$	$202 * 10^5$	$190 * 10^5$
Faccal streptococci Count/ 100ml	$290 * 19^2$	$220 * 10^2$	$203.25 * 10^2$

protein food for people, recycling of sewage water for culturing of fishes might add to human welfare and material benefits.

The enterprise, thus turns out be economically viable and remunerative. This should enthuse the planners incharge of administration to design and execute suitable wastewater disposal and stabilization systems, wherever feasible like civic bodies, housing colonies and educational institutions, for realizations of the twin objectives, namely hygienic disposal, freeing the environment from the pollutional hazards and recycling of domestic wastewaters for economic gains as well, besides contributing to protein-rich fish food.

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