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Enteric Bacteria Associated with Farmed Freshwater Fish and its Culture Environment in Kerala, India

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Abstract: A study was designed to investigate the enteric bacterial population associated with farmed freshwater fish and its environment, limnological quality of carp farm and the existing association between these parameters. Enteric indicator bacterial counts were determined following the United States Food and Drug Administration (USFDA) methods and the physico-chemical parameters according to the standard methods of American Public Health Association (APHA). Fish samples yielded mean microbiological counts in the range of 4.19 to 4.85 log CFU g^{-1}, sediment in the range of 5.18±0.01 to 6.34±0.01 log CFU g^{-1}, pond water in the range of 3.64±0.03 to 6.10±0.04 log CFU mL^{-1}. Fish and feeder canal water showed higher count for all indicator bacterial count. Sediment showed 2 log cycle higher count of sulphite reducing *clostridia*. Emerging pathogen *E. coli* O157:H7 were absent in all the samples analyzed. *Aeromonas* (26.2%) followed by *Enterobacter* (24.6%) were the dominant flora recovered. *Escherichia*, *Klebsiella*, *Serratia*, *Hafnia*, *Plesiomonas*, *Shigella*, *Salmonella*, *Morganella* and *Yersinia* were the other opportunistic enteric bacterial pathogens detected from this system. The rearing practices such as natural fertilization and feeding could have influenced the enteric flora. Study on the various physico-chemical parameters of pond water revealed that they were within the suitable range for the freshwater fish culture throughout farming phase. Correlation analysis revealed a significant positive correlation between physico-chemical parameters such as total organic carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) with that of Total Plate Count (TPC), Total *Enterobacteriaceae* Count (TEC), Total Coliforms (TC), Faecal Coliforms (FC) and *E. coli* (EC). Presence of bacteria of public health significance in the aquaculture ponds envisages a strict hygienic handling and processing of fish from the culture systems for ensuring public health safety.

Key words: Freshwater fish, aquaculture environment, enteric bacteria, water quality

INTRODUCTION

India witnessed an overwhelming growth of the aquaculture sector during the last two decades and ranked second in aquaculture production. Carp culture plays an indispensable role with a percentage contribution of 93.6 to freshwater aquaculture production of India

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(FAO, 2005). While this growth is much appreciated in terms of food security, the health risk associated with the aquaculture produce is another important concern. 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 of fish by pathogens is likely to occur, when the fish are raised in ponds with faecal coliforms, *E. coli* and *Salmonella* of greater than 10^4, 10^{3-4} and 10^5 per 100 mL in pond water, respectively (Guzman *et al.*, 2004). Ogbondeminu and Okoeme (1989) reported that 50% of the microorganisms recovered from both fish and water of earthen 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 environment (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 were also increasing (Samadpour *et al.*, 1994; Kumar *et al.*, 2001; Teophilo *et al.*, 2002).

The microbial shifting with reference to indicator quality and enteric bacteria and their relationship with water quality during farming phase was an unexplored area in carp farm. Hence, it is essential to investigate the composition of enteric flora associated with carp grow-out culture in order to develop safe farm management practices for the production of carp safe for human consumption (Reilly and Kaferstein, 1999). Thus, the aim of the present study is to investigate the enteric bacterial population associated with farmed freshwater fish and its environment, limnological quality of carp farm and the existing association between this parameter. The information obtained should allow a better control of the bacteriological parameters in the aquacultured carps and also a better control of processing to reduce the possible microbial risks.

MATERIALS AND METHODS

The pond chosen for the study was a composite carp culture farm situated in Thiruvankulam, Kerela, India. This is an earthen pond having an area of 0.4 ha with a stocking density of 10,000 fingerlings ha^{-1} at a ratio of 3: 4:1:2 for catla, rohu, mrigal and grass carp, respectively. The pond was surrounded by two rearing pond in two sides, third and fourth side was covered by feeder canal and a freshwater body. Pond dykes were covered with grass and pond bottom was silty. The pond was fertilized with natural manure (cow dung) initially and continued every 15 days according to the nutrient status. Feeding was by farm made feeds prepared out of rice bran and groundnut oil cake. There was periodic water exchange by pumping the water from the feeder canal, which was always covered with aquatic weeds like pistia, lemna, cerotopyllum, etc. and contained aquatic animals like snails.

Sampling

Four sampling was done with a periodic interval of 2 months to cover the culture period of eight months. Fish samples ten each in a sampling period were harvested by cast net and put in sterile polythene bag. Water samples were collected from four different location of the pond by inverting the sterile polystyrene bottle to about 30 cm below the surface and a well-mixed homogeneous sample was used for analysis. Feeder canal water samples were collected in the same manner. Pond sediment were scooped out from four different locations of the pond and collected in a sterile polythene bag. All the samples were kept in icebox

carried to laboratory. Analysis was initiated within 2 h of sample collection. Sampling was done between 08: 00 to 09: 00 h Indian Standard Time (IST).

Water Quality Parameters

Water temperature, pH and salinity were determined by thermometer, digital pH meter (Cyberscan, USA) and refracto meter, respectively at the farm location. Transparency was measured using Secchi disc (Trivedy and Goel, 1984). Total Suspended Solids (TSS) and Dissolved Oxygen (DO) were analysed according to the standard methods for examination of water and wastewater (APHA/AWWA/WEF, 1998). TOC, NO_3-N (nitrate nitrogen), BOD, COD, Surfactant, were measured by properly calibrated pastel UV spectrophotometer (SECOMAM RS 232, France) with a spectral range of 200 to 320 nm. Turbidity was measured by employing the digital turbidity meter (Merck, USA). Conductivity of the water was measured by using electronic conductivity meter (CIFT, India).

Bacteriological Analysis

Ten grams of the muscle portion of fish along with skin and sediment samples were homogenized for 1 min with 90 mL of saline (0.85% NaCl) in a stomacher 400 lab blender (Seward, London, UK). Fish, sediment and water samples were serially diluted and used for analysis. The total plate count was enumerated by pour plating the samples on tryptone glucose agar (TGA), Total *Enterobacteriaceae* count on violet red bile glucose agar (VRBGA, Oxoid, Basingstoke, UK) and faecal *Streptococci* in Kenner Faecal streptococcus Agar (KF) (USFDA, 2001).

Total coliforms, faecal coliforms and *E. coli* counts were estimated by a three-tube/five tube most-probable number (MPN) procedure for fish/sediment and water samples, respectively with the following modification. Aliquots of serially diluted samples were inoculated into MacConkey broth (Oxoid, UK) and incubated at 37°C for 24-48 h. Positive tubes were (1) subjected to an MPN procedure in brilliant green lactose bile broth (Oxoid, UK) at 37°C for 24-48 h and (2) subjected to elevated coliform (Difco, BD, Frankline Lakes, NJ, USA) and indole (Difco, USA) broths at 44.5°C. Positives in Brilliant Green Lactose Broth (BGLB), elevated coliform (EC) broth and Indole were noted down and referred to McCardy's MPN table to determine the total coliforms, faecal coliforms and *E. coli* counts, respectively. A loopful of bacteria culture from indole positive tubes was streaked on eosine methylene blue agar (EMB agar, BBL, BD, Sparks, Md.) and characteristic *E. coli* colonies were isolated and confirmed by indole-methyl red-Voges-Proskauer-citrate (IMViC) tests (APHA/AWWA/WEF, 1998). Sulphite reducing *clostridia* were analysed by MPN method using differential reinforced clostridia medium (DRCM) (USFDA, 2001).

Detection of *E. coli* O157:H70

Twenty five grams of the fish muscle with skin, sediment and 25 mL of water samples were enriched in 225 mL of modified elevated coliform broth (Difco, USA) containing novobiocin (20 mg mL^{-1}; Sigma, St. Louis, Mo.) at 42°C with shaking (150 rpm). After an overnight incubation, diluted enrichment samples were plated on MacConkey sorbitol agar (Oxoid, UK) supplemented with cefixime (SR 0191, Oxoid, UK) and potassium tellurite (March and Ratman, 1986). Plates were incubated overnight at 37°C and sorbitol-negative colonies were isolated at a rate of two to three colonies per sample. After isolate purification, they were streaked on EMB agar and confirmed isolates were checked for MUG (methylumbelliferyl-β-glucuronide) reaction and IMViC test. MUG and sorbitol-positive *E. coli* (ATCC 25922) were used as controls for checking sorbitol and MUG reactions.

Isolates that were sorbitol and MUG negative were tested for latex agglutination with *E. coli* serotype O157 specific antisera as per manufacturer's instruction (Oxoid, UK).

Characterization of Enteric Bacteria

For identification 2-5 well-separated typical colonies from VRBGA plates were selected using Harrison's disc method (Harrigan and McCance, 1976). These cultures were purified and stored for further study in nutrient agar slants. Altogether, 130 pure culture isolates were obtained and identified up to the genus level with the help of an identification scheme from web http//www.vet.uga.edu/WEBFILES/ in consultation with Edwards and Ewing (1972) and MacFaddin (1980). About 5% of the isolates were crosschecked for identification using analytical profile index 20E (API 20E, bioMerieux).

Statistical Analysis

All the results were produced as mean and mean log±standard deviation values for water quality and microbiological parameters, respectively. Statistical analysis between the means was accomplished using tukey's test and a two-tailed Pearson's correlation analysis was carried out. The statistical package used in the study is SPSS, 10.

RESULTS AND DISCUSSION

Physico-chemical Characteristics of Water

The range of temperature and pH recorded for pond water were 28.10±0.26 to 30.8±0.20 and 6.15±0.03 to 7.84±0.02, respectively (Table 1). The maximum salinity in both pond (0.20 ppt) and feeder canal water (0.23 ppt) was during last phase of sampling which fell on summer months. The temperature, pH, salinity and NO_3-N recorded in the pond and feeder canal water were within the desirable range for freshwater fish culture (IS 13891, 1994) and comparable to earlier reported values from tropical fish ponds (Pilarski *et al.*, 2004; El-Shafai *et al.*, 2004). El-shafai *et al.* (2004) reported a very low level of TSS 5±2 in tilapia pond. However, in this study it was much higher and reached up to 62.67±2.02 and 83.33±1.16 in pond and feeder canal water, respectively. Surfactants in the fishpond should not form any film and obstruct fish growth (IS 13891, 1994). Though surfactants were detected in pond and comparatively higher quantities in feeder canal water, they were not to the extent of obstructing fish growth. As culture period progresses, TOC, BOD, COD, Turbidity (except third quarter) showed an increasing trend and DO showed a decreasing trend in pond water.

Table 1: Physiochemical parameters of pond and feeder canal water quality of rohu fish farm

	Pond water				Feeder canal water			
Parameters	Phase I	Phase II	Phase III	Phase IV	Phase I	Phase II	Phase III	Phase IV
Temp (°C)	30.8±0.20^{a}	28.10±0.26^{c}	29.93±0.12^{b}	28.23±0.21^{c}	28.83±0.29^{a}	28.27±0.15^{b}	28.93±0.12^{a}	28.13±0.12^{b}
pH	6.20±0.03^{c}	6.36±0.03^{b}	7.84±0.02^{a}	6.15±0.03^{c}	5.42±0.03^{d}	6.26±0.02^{c}	7.43±0.02^{a}	6.44±0.05^{b}
Salinity (ppt)	0.03±0.06^{c}	0.10±0.00^{bc}	0.13±0.06^{bc}	0.20±0.00^{b}	0.07±0.06^{a}	0.17±0.06^{a}	0.20±0.10^{a}	0.23±0.06^{a}
TSS (ppm)	46.50±0.50^{b}	62.67±2.02^{a}	41.17±1.26^{c}	38.67±2.40^{c}	83.33±1.16^{a}	78.23±1.75^{b}	8.43±0.15^{d}	48.37±2.01^{c}
TOC (ppm)	3.97±0.15^{d}	5.33±0.25^{c}	6.33±0.12^{b}	7.53±0.15^{a}	5.50±0.10^{b}	4.60±0.17^{d}	5.03±0.02^{c}	10.30±0.10^{a}
NO_3-N (ppm)	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	1.60±0.00^{a}	1.40±0.00^{b}
Surfactant (ppm)	1.27±0.06^{c}	2.17±0.06^{a}	1.83±0.06^{b}	Less than 0.2	2.53±00.06^{a}	2.03±0.15^{b}	Less than 0.2	2.20±0.10^{b}
COD (ppm)	11.97±0.15^{d}	14.90±0.50^{c}	19.10±0.36^{b}	24.80±0.36^{a}	13.57±0.21^{b}	11.00±0.26^{c}	13.00±0.20^{b}	28.53±0.50^{a}
BOD (ppm)	5.57±0.15^{d}	7.30±0.26^{c}	9.40±0.17^{b}	11.13±0.31^{a}	6.13±0.15^{b}	4.83±0.21^{c}	6.00±0.10^{b}	13.60±0.20^{c}
Turbidity (NTU)	24.33±0.58^{b}	25.00±0.00^{b}	20.67±0.58^{c}	29.67±0.58^{a}	41.33±0.58^{b}	28.67±0.58^{c}	10.33±.58^{d}	44.67±0.58^{a}
SDT (cm)	25.83±0.76^{c}	27.00±0.87^{c}	41.00±0.50^{b}	45.17±0.76^{a}	ND	ND	ND	ND
Conductivity (milli mhos)	0.46±0.01^{b}	0.36±0.06^{b}	-62.77±0.75^{c}	21.77±0.55^{a}	0.24±0.00^{b}	0.31±0.045^{b}	-40.47±0.36^{c}	11.53±0.25^{a}
DO (ppm)	4.69±0.19^{a}	4.07±0.08^{b}	3.87±0.04^{b}	3.28±0.07^{c}	3.40±0.05^{a}	0.89±0.00^{c}	3.53±0.046^{a}	1.26±0.16^{b}

Where, TSS-Total suspended solids, TOC-Total organic carbon, COD-Chemical oxygen demand, BOD-Biological oxygen demand, SDT-Secchi disc transparency, DO-Dissolved oxygen ppt-parts per thousand, ppm-parts per million, NTU-Neplometric turbidity unit, cm-centimetre, Results are presented as mean ± standard deviation (SD), ^{a,b,c,d}Means in a column with the same superscript letters are not significantly different (p>0.05)

This may be due to the rise in the level of fish biomass towards the end of the culture (Markosova and Jezek, 1994; Komarkova *et al.*, 1986). The range of TOC, COD, BOD, Turbidity and DO in pond water were 3.97±0.15 to 7.53±0.15 ppm, 11.97±0.15 to 24.80±0.36 ppm, 5.57±0.15 to 11.13±0.31 ppm, 20.67±0.58 to 29.67±0.58 NTU and 3.28±0.07 to 4.69±0.19 ppm, respectively. They were significantly different ($p<0.05$) in different quarters of culture period (Table 1). Other studies recorded a slightly higher DO for carp and Tilapia ponds in pond water (Pilarski *et al.*, 2004; El-shafai *et al.*, 2004). The turbidity levels were always well above the tolerable level for freshwater fish culture of 10 NTU (IS 13891, 1994) but similar to level recorded in pig manured (45 to 65 ppm) and non manured (35 to 25 ppm) common carp pond (Pilarski *et al.*, 2004). A similar trend as above was absent in feeder canal water and showed high fluctuations among different quarters. Conductivity was maximum in the last sampling and a similar trend was noticed both water samples.

Microbial Quality of Fish and Pond Environment

The microbial quality of the samples of fish, sediment, feeder canal and pond water at four different stages of culture period are shown in the Table 2. The TPC of the above samples ranged between 3.64±0.03 and 6.47±0.03 log CFU g^{-1}. The range obtained for fish samples were 4.19 to 4.85 log CFU g^{-1} (Table 2). A TPC of 3 to 5 log CFU g^{-1} was widely documented for carp samples from different part of the world (Sugita *et al.*, 1985; Ogbondeminu, 1993; Jeyasekaran and Ayyappan, 2003). However, the present level of TPC for carp muscle with skin was 1-2 log CFU g^{-1} lower than the reported TPC for gill and intestines of carp samples (Nedoluha and Westhoff, 1997; Hatha *et al.*, 2000). Sediment samples showed 1-2 log CFU g^{-1} higher TPC values than fish and water samples (3.64±0.03 to 6.47±0.03 log CFU mL^{-1}) as reported in earlier studies (Sugita *et al.*, 1985; Jun *et al.*, 2000).

In general indicator organisms viz., Total *Enterobacteriaceae* (TE), Total Coliforms (TC), Faecal Coliforms (FC) and *E. coli* (EC) were recorded highest in feeder canal water followed

Table 2: Microbiological quality of rohu fish farm during different phase of culture

	Samples							
	TPC	TEC	TCC	FCC	ECC	FSC	SRCC	*E. coli*O157
Fish	(log CFU g^{-1})		(log MPN g^{-1})			(log CFU g^{-1})		
Phase I	4.19±0.01^{d}	3.93±0.04^{a}	3.45±0.05^{a}	2.64±0.03^{a}	2.37±0.06^{a}	Less than 2	2.59±0.10^{b}	Less than 2
Phase II	4.74±0.03^{b}	2.73±0.01^{d}	1.57±0.15^{d}	1.07±0.04^{c}	0.96±0.05^{d}	2.51±0.05^{b}	2.19±0.02^{c}	Less than 2
Phase III	4.66±0.02^{c}	3.34±0.02^{c}	2.44±0.13^{c}	2.43±0.05^{b}	1.95±0.06^{c}	1.91±0.06^{c}	3.19±0.02^{a}	Less than 2
Phase IV	4.85±0.01^{a}	3.41±0.01^{b}	2.83±0.04^{b}	2.68±0.04^{a}	2.12±0.02^{b}	2.92±0.01^{a}	3.21±0.03^{a}	Less than 2
Sediment	log CFU g^{-1}	log CFU g^{-1}	log MPN g^{-1}	log MPN g^{-1}	log MPN g^{-1}	log CFU g^{-1}	log CFU g^{-1}	log CFU g^{-1}
Phase I	5.38±0.02^{c}	2.37±0.01^{c}	1.65±0.03^{a}	1.12±0.51^{a}	0.14±0.02^{b}	Less than 2	2.64±0.03^{c}	Less than 2
Phase II	6.34±0.01^{a}	3.30±0.04^{a}	0.99±0.05^{b}	0.71±0.02^{ab}	0.70±0.04^{a}	1.07±0.04^{a}	3.54±0.03^{b}	Less than 2
Phase III	6.07±0.02^{b}	2.49±0.04^{b}	0.17±0.05^{c}	0.14±0.02^{bc}	0.12±0.02^{b}	Less than 2	4.12±0.07^{a}	Less than 2
Phase IV	5.18±0.01^{d}	Less than 2	ND	ND	ND	Less than 2	3.64±0.03^{b}	Less than 2
PW	log CFU mL^{-1}	log CFU 100 mL^{-1}	log MPN 100 mL^{-1}			log CFU mL^{-1}		
Phase I	3.64±0.03^{d}	2.36±0.04^{c}	1.68±0.05^{d}	1.54±0.06^{c}	1.50±0.04^{c}	Less than 2	2.57±0.05^{d}	Less than 2
Phase II	4.06±0.02^{c}	2.59±0.05^{b}	2.07±0.04^{c}	1.78±0.05^{b}	1.58±0.05^{c}	Less than 2	2.88±0.12^{c}	Less than 2
Phase III	4.45±0.01^{b}	2.66±0.02^{b}	2.25±0.05^{b}	1.91±0.06^{b}	1.84±0.02^{b}	Less than 2	3.12±0.14^{b}	Less than 2
Phase IV	6.10±0.04^{a}	2.91±0.08^{a}	2.40±0.04^{a}	2.06±0.02^{a}	2.03±0.06^{a}	Less than 2	3.56±0.03^{a}	Less than 2
FCW	log CFU mL^{-1}	log CFU 100 mL^{-1}	log MPN 100 mL^{-1}			log CFU mL^{-1}		
Phase I	4.14±0.01^{b}	3.61±0.05^{b}	2.70±0.05^{c}	2.51±0.06^{b}	2.37±0.05^{b}	2.68±0.05^{a}	3.38±0.03^{a}	Less than 2
Phase II	3.51±0.03^{c}	2.62±0.03^{d}	2.00±0.04^{d}	1.65±0.03^{c}	1.56±0.05^{c}	1.13±0.10^{c}	3.24±0.01^{ba}	Less than 2
Phase III	4.09±0.05^{b}	3.19±0.06^{c}	3.12±0.14^{b}	2.56±0.03^{b}	2.51±0.06^{b}	Less than 2	3.12±0.14^{b}	Less than 2
Phase IV	6.47±0.03^{a}	4.47±0.02^{a}	3.40±0.17^{a}	3.20±0.05^{a}	3.12±0.14^{a}	2.14±0.09^{b}	3.37±0.06^{a}	Less than 2

Results are presented as mean ± standard deviation (SD).^{a,b,c,d} Means in a column with the same superscript letters are not significantly different ($p>0.05$),TPC-Total Plate Count, TEC-Total *Enterobacteriaceae* count, TCC-Total coliforms count, FCC-Faecal coliform count, ECC-*E. coli* count, FSC-Faecal streptococci count, SRCC-Sulphite reducing clostridia count

by fish, pond water and sediment (Table 2). The range recorded for TE, TC and FC, respectively are 0 to 4.47±0.02, 0 to 3.45±0.05 and 0 to 3.20±0.05 log CFU mL^{-1}. These results are comparable or one log cycle lower than the natural manure fertilized fish culture ponds stocked with carps, tilapia etc. (Quines, 1988; Pilarski *et al.*, 2004). Surendran *et al.* (1995) observed consistently elevated levels of TE, TC and FC in freshwater fish and shellfish than brackish water and marine fishes and concluded that these were native flora of cultured freshwater fish and prawn.

All the analyzed samples except one sediment sample were contaminated with *E. coli*. However, earlier studies reported a comparatively low level of contamination by *E. coli* (0 to 50% of the samples) on carps from India (Nair and Nair, 1988; Jeyasekaran and Ayyappan, 2003). Feeder canal showed a higher level of all microbial parameters due to the presence of aquatic weeds, aquatic animals like snails and run off from near by agriculture land, which are potential source of contamination (Ferguson *et al.*, 1996; Islam *et al.*, 2000).

Faecal *Streptococci* (FS) were detected in fish (less than 2 to 2.92±0.01 log CFU g^{-1}) and feeder canal water samples (range less than 2 log CFU g^{-1} to 268±0.05 log CFU mL^{-1}). It was less than 2 log CFU g^{-1} in pond water and was detected only in second phase of culture in the case of sediment samples. This was in accordance with an earlier study (Niemi and Taipalinen, 1982). The authors reported a very low count in sediment. All the enteric bacterial counts were comparatively lower in pond water. It was reported that sunlight play a significant role in their survival, which was prominent in pondwater than feedercanal water due to higher surface area and absence of vegetation in pondwater (Ferguson *et al.,* 1996). Sulphite reducing *clostridia* was detected in all samples and was high in sediment samples followed by feeder canal water, pond water and fish (Table 2). This may be due to the prevalence of anaerobic condition in the sediments and sediment was reported as potential reservoir for aquatic bacteria especially anaerobic bacteria like SRC (Ferguson *et al.,* 1996). Though there are reports of occurrence of pathogenic strains of *E. coli* from market seafood from different part of the world (Samadpour *et al.*, 1994; Teophilo *et al.*, 2002) and India (Kumar *et al.*, 2001), it is clear from the findings of the present study that all samples of fish, sediment and water from fresh water fish farm studied were free from the emerging pathogen *E. coli* O157:H7.

An increasing trend for almost all the microbial parameters as culture progresses was noticed in fish (from phase II onwards) and both water samples. Increased fish biomass, accumulation of fish faeces and leftover feed in the earthen pond may have contributed to the higher count towards at the end (Davis and Goulder, 1993; Markosova and Jezek, 1994). The first phase of sampling was during a heavy rainy season and hence surface run off might have contributed to the abnormally high count.

Composition of Enteric Bacteria in Carp Farms

Characterization of 130 enteric bacterial isolates from carp farm revealed that *Aeromonas* sp. (26.2%), a non *Enterobacteriaceae* member and common contaminant of fish and its environment (Thampuran and Surendran, 1998; Apun *et al.*, 1999; Hatha *et al.*, 2000), was the dominant flora. This was closely followed by *Enterobacter* sp. (24.6%). Other genera recovered include *Escherichia*, *Klebsiella*, *Serratia*, *Hafnia*, *Plesiomonas*, *Shigella*, *Salmonella*, *Morganella* and *Yersinia*. Recovery of *Aeromonas* sp. as dominant genera in this study was of concern for well beings of fish, as it was established as the secondary infecting agent in rohu fingerlings (Sugumar *et al.*, 2002). Fish samples harbored the diverse group of bacteria and as many as 8 genera were detected. *Escherichia/Aeromonas*, *Klebsiella*, *Aeromonas* and *Enterobacter* were the dominant flora of fish, feed,

Table 3: Composition of enteric bacteria among different samples

	Samples											
	Fish		Mud		PW		SW		FD		Total	
Groups	No.	%	No.	-	No.	%	No.	%	No.	%	No.	%
Aeromonas	10	27.8	10	55.6	8	25	6	16.7	-	-	34	26.2
Enterobacter	6	16.7	-	-	8	25	16	44.4	2	25	32	24.6
Escherichia	10	27.8	2	11.1	4	12.5	4	11.1	-	-	20	15.4
Hafnia	2	5.6	-	-	-	-	4	11.1	-	-	6	4.6
Klebsiella	-	-	-	-	2	6.3	-	-	6	75	8	6.2
Morganella	2	5.6	-	-	2	6.3	-	-	-	-	4	3.1
Plesiomonas	2	5.6	4	22.2	-	-	-	-	-	-	6	4.6
Salmonella	-	-	2	11.1	-	-	2	5.6	-	-	4	3.1
Serratia	2	5.6	-	-	2	6.3	4	11.1	-	-	8	6.2
Shigella	-	-	-	-	6	18.8	-	-	-	-	6	4.6
Yersinia	2	5.6	-	-	-	-	-	-	-	-	2	1.5
Unidentified	-	-	-	-	-	-	-	-	-	-	-	-
Total	36	100	18	100	32	100	36	100	8	100	130	100

Note: PW-Pond water, SW-Seawater, FD-Feed

sediment and water samples, respectively (Table 3). The present results were similar to earlier studies on Cyprinid and other fishes and their environment (Niemi and Taipalinen, 1982; Apun *et al.*, 1999; Ampofo and Clerk, 2003). *Escherichia* which was recovered from all the samples except feed in the present study and was reported as predominant enteric bacteria from intestines of freshwater fishes (carp, tilapia and catfish) and pond water (Niemi and Taipalinen, 1982; Ogbondeminu, 1993). *Citrobacter*, *Proteus*, isolated in earlier studies (Niemi and Taipalinen, 1982; Apun *et al.*, 1999) were absent in this study.

There are reports of isolation of different members of *Enterobacteriaceae* viz., *Edwardsiella ictaluri*, *E. tarda*, *Proteus roettgeri*, *Yersinia ruckeri*, *Erwinia* sp., *Serratia*, *Citrobacter freundii*, *Aeromonas*, *Salmonella*, *Shigella* and *Yersinia pseudotuberculosis*, as potential fish and human pathogens from natural manured carps, stripped bass, tilapia, eel and its earthen culture environment (Nair and Nair, 1988; Karunasagar *et al.*, 1992; Nedoluha and Westhoff, 1997; Muratori *et al.*, 2000) and their occurrence was much higher in integrated fish culture systems (Ogbondeminu, 1993). In the present study *Salmonella* and *Shigella* was recovered from sediment and water samples only and others were absent. Though fish was free from all the above pathogens (except *Yersinia*), there exist an inherent risk of contamination by pathogens present in environment as observed in natural manured earthen ponds (Nedoluha and Westhoff, 1997). The results indicated that *Aeromonas* and *Enterobacter* were recovered through out the farming phase and highest recovery was in second and fourth quarter, respectively. *Escherichia* was not detected in the second quarter and were consistently present in all other quarters. Potential human pathogens *Shigella, Yersinia, Klebsiella* and *Morganella* were recovered only in third and fourth quarters except in the case of *Salmonella.* Maximum diversity of enteric bacteria was noticed in third phase. (Table 4).

Correlation Between Physico-chemical and Microbial Parameters

The relationship between physico-chemical parameters and bacterial count attracted much of attention (Ogbondeminu and Adeniji, 1984; Guo *et al.*, 1988; Ferguson *et al.,* 1996). In this study correlation between freshwater pond microbiological quality and physic-chemical parameters was established using the two tailed Pearson's correlation coefficient ($p<0.05$ and 0.01). It was observed that water temperature showed a negative effect on all bacterial indicator parameters analysed except EC and FS in pond water ($p<0.05$).

Table 4: Changes in the composition of enteric bacteria during rohu farming

	Farming phase									
	I		II		III		IV		Total	
Group	No.	%	No.	-	No.	%	No.	%	No.	%
Aeromonas	4	13.3	14	46.6	8	20	8	20	34	26.2
Enterobacter	8	26.6	4	13.3	8	20	12	30	32	24.6
Escherichia	8	26.6	-		6	15	6	15	20	15.4
Hafnia	2	6.7	2	6.7	2	5	-	-	6	4.6
Klebsiella	-	-	-	-	8	20	-	-	8	6.2
Morganella	-	-	-	-	4	10	-	-	4	3.1
Plesiomonas	-	-	6	20	-	-	-	-	6	4.6
Salmonella	4	13.3	-	-	-	-	-	-	4	3.1
Serratia	4	13.3	4	13.3	-	-	-	-	8	6.2
Shigella	-	-	-	-	2	5	4	10	6	4.6
Yersinia	-	-	-	-	2	5	-	-	2	1.5
Unidentified	-	-	-	-	-	-	-	-	-	-
Total	30	100	30	100	40	100	30	100	130	100

Similarly, Ferguson *et al.* (1996) reported the killing effect of temperature on various indicator bacterial parameters. Conversely there were reports (Sugita *et al.*, 1985; Markosova and Jezek, 1994) showing that populations of indicator bacteria increased with increasing water temperature as temperature become favorable for growth of bacteria during summer months. TOC, BOD and COD indicates directly or indirectly the organic pollution status and a change in above of pond and feeder canal water positively affected TPC, Total coliforms, Faecal coliforms, *E. coli* count (significant at $p<0.05$ and 0.01). It was reported that organic matter helps in greater survival of aquatic bacteria (Gerba and McLeod, 1976) and their increase in water lead to the significant increment in density of FC and FS (Sugita *et al.*, 1985; Ferguson *et al.*, 1996).

Similar to an earlier observation involving estuary water (Ferguson *et al.*, 1996), in this study also turbidity showed a positive correlation to pond water TPC ($p<0.05$). Pal and Das Gupta (1992) established that environment could influence the microflora of the fish and pond systems. Several authors showed a significantly positive correlation between TPC of pond water and fish (Ogbondeminu, 1993; Apun *et al.*, 1999). In the present study pond water microbial parameters TPC, TE, TC, FC, EC and SRC showed a significant positive correlation with fish TPC ($p<0.01$), FS ($p<0.01$) and SRC ($p<0.05$).

CONCLUSIONS

The enteric bacterial counts were detected in higher numbers and some of the members recovered in this study like *Salmonella*, *Shigella* and *Yersinia* were established fish/human pathogens. *E. coli* has an assertive existence in carp ponds, which is evident from their wide spread prevalence across farming phase and among all samples analysed. Further study on the antibiotic resistance profile of these enteric pathogens could establish the real threat posed by these organisms. However, detection of diverse group of enteric bacteria including potential pathogens in the carp culture ponds suggests that strict hygiene procedures should be followed during handling and processing of fish from the similar culture systems and proper cooking prior to consumption to prevent the transfer of potentially pathogenic bacteria to humans. Good correlation between the bacterial population and the water quality variables like TOC, BOD, COD opens up an avenue for research in this line to establish a water quality and microbial quality model useful in assessing the quality and safety status of farm raised fish.

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