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Microbial Hazard Analysis of Fish (*Catla Catla*) at Various Stages of Supply Chain

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ABSTRACT

The present study was planned with the objectives to evaluate microbial quality of freshwater fish (*Catla catla*) sold in retail market in Parbhani city at harvest, transportation and fish shop. A total of 18 fish samples were collected as per method prescribed by ICMSF (1998) and analyzed. The per cent *Staphylococcus aureus* positive samples found were 50 per cent at harvest, 83.33 per cent during transportation and 100 per cent at retail shop. The *E.coli* isolations at various stages of supply like harvest, transportation and retail were 33.33 per cent, 50 per cent and 83.33 per cent respectively. The *Salmonella* spp isolations were 16.66 per cent at harvest, 83.33 per cent at transportation and 50 per cent at retail. The *Clostridium* spp isolations were seen in 33.33 per cent samples at harvest, 50 per cent during transportation and 100 per cent at retail shop. The *Vibrio* spp isolation at harvest, transportation and retail shop were 100 per cent, 83.33 per cent and 33.33 per cent. Identification of *Salmonella* spp and *Vibrio* spp indicate concern from public health point of view. Presence of various pathogenic micro-organisms at various stages of fish supply chain indicates sources of contamination in environment and need sanitary measures.

Keywords: *Catla catla*, public health, bacteriological, parbhani

In recent times quality assessment of food at different operational levels is becoming essential to achieve one health objective set by World Health Organization. Rapid urbanization is leading to increasing demand for food products. Quality awareness of consumers from availability of technologies like cold chain, faster transportation and quality standards are making fish industry more dynamic and efficient. In semi-urban areas fish is being sold by traditional methods which are being sourced from local resources. *Catla catla* is becomed the popular fish due to its taste and economic (Hasan *et al.*, 2012).

Fish contain fat free amino acids and water which is susceptible to spoilage by micro-organisms and biochemical reaction during post-mortem process. Fish are perishable and can be kept fresh in ice for 8-14 days. The maintenance of cold condition from harvest to retail shop is of paramount importance in relation to quality management (Rehman *et al.*, 2003). Fish sold at open market and exposed to ambient temperature increases likelihood of spoilage. Marketing and handling chain involving fresh fish is important from public health point of view (Kapute *et al.*, 2012).

The reservoir at Yelderi on Purna River is situated in Parbhani district of Maharashtra. The reservoir water of dam is used for carp culture of *Catla catla* on co-operative basis. The harvest is being sold in Parbhani retail market which is around 60 km from reservoir. Holistic evaluation of entire fish culture operation is important from public health point of view (Sherikar *et al.*, 2013). Hence the present study is based on the objective to assess microbial quality of freshwater fish (*Catla catla*) sold in retail market in Parbhani city at harvest, transportation and fish shop.

MATERIALS AND METHODS

Fish samples were collected for microbial studies from harvest at Yelderi dam reservoir, during transportation and at retail market in Parbhani city. A total of 18 samples of fish (6 samples of harvest, 6 samples during transport and 6 samples at retail shop) were collected in sterile polythene

Table 1: Isolation of Bacterial pathogens from fish at various stages of supply chain

Stages of Supply Chain	Percenatges of *Staphylococcus aureus*	Percenatges of *E. coli*	Percenatges of *Salmonella* spp.	Percenatges of *Clostridium* spp	Percenatges of *Vibrio* spp	Percenatges of Yeast & Molds
Harvest	50.00	33.33	16.66	33.33	100	0.00
Transportation	83.33	50.00	83.33	50.00	83.33	50.00
Retail Shop	100.00	83.33	50.00	100.00	33.33	100.00

bags as per the method described by ICMSF (1998). The samples were transported in ice box to the laboratory.

Isolation of *Staphylococcus aureus, E. coli*, *Salmonella* spp, *Clostridium* spp, *Vibrio* spp and yeast and moulds were done as per the method described in BAM (1998) with selective agar medium. All the agar media were used from Hi-Media Laboratories, Mumbai. A total of 0.1 ml inoculums of 10^{-3} and 10^{-4} dilutions of sample on Vogel Johnson (VJ) which was spread by means of L-shape spreader and plates were kept overnight at 37ºC for incubation for isolation of *Staphylococcus aureus*. For isolation and counting of *E. coli* 0.1 ml of inoculom from dilution of 10^{-3} and 10^{-4} were used by spread plate technique on Levine's Eosine Methylene Blue Agar (EMB). For isolation of *Salmonella* spp pre-enriched sample was inoculated in tetra-thionate broth (TTB) and incubation was done for 24 hours at 42ºC and inoculums was streaked over Xylose Lysine Deoxycholate (XLD agar. Sodium Polymixin Sulpha-diazine (SPS) agar was used for isolation of *Clostridium spp*. Alkaline peptone water (APW) was used as enrichment medium for *Vibrio* spp isolation. At the end of incubation period loop of culture from enriched broth APW was taken and streaked over thiosulfate citrate bile salts sucrose (TCBS) agar plates. A total of 0.1 ml. inoculums of 10^{-3} and 10^{-4} dilutions of sample on Sabouraud Dextrose Agar by spread plate method for isolation and counting of yeast and molds. The data generated in the study was analyzed as per the method Snedecor and Cochran (1967).

RESULTS AND DISSCUSSION

The aquatic medium is extremely vulnerable to pollution and that could result into microbial contamination. The public health significance of fish contamination lies not only in their ability to cause diseases but also their possible role in transfer of antibiotic resistance strain to other pathogens. In view of this fact present study was designed to assess the microbial quality of fish in various supply chain with respect to isolation of pathogens like *Staphylococcus aureus, E.coli*, *Salmonella* spp, *Clostridium* spp, *Vibrio* spp and yeast and moulds.

In present study, fish samples were screened for *Staphylococcus aureus* on VJ agar medium at various stages of supply chain. The samples were recorded as per cent positive upon growth of characteristics colonies on VJ agar medium. The percentage of *Staphylococcus aureus* isolation found were 50.00 per cent of fish at harvest, 83.33 per cent during transport and 100.00 per cent at retail shop. The results are given in Table 1. The sources of *Staphylococci* in subsequent stages of supply chain were due to contamination during handling and transportation (Jayasekaran and Ayyappan, 2003). The mean *Staphylococcus aureus* count were found to be 5.36 ± 0.12 $\log_{10}$ CFU/gm in fish at harvest, 5.71 ± 0.12 $\log_{10}$ CFU/gm during transport and 5.80 ± 0.12 $\log_{10}$ CFU/gm at retail shop. The results are depicted in Table-2. Statistical analysis of mean *Staphylococcus aureus* count with help of "t" test revealed non-significant differences thereby indicating no effect of stages of sample collection upon *Staphylococcus aureus* counts. The present observation was in agreement with Pamuk *et al*. (2011) who observed *Staphylococcus* count of 10^6 to 10^7 cfu/ml in carp fishes.

E. coli were isolated on EMB agar for primary isolation and were enumerated for differential counts from fish samples collected at various stages of supply chain. The percentage of *E.coli* was found to be 33.33 per cent at harvest followed by 50.00 per cent during transportation and 83.33 per cent at retail shop. The results are depicted in Table 1. In present study *E.coli* counts recorded were 5.79 ± 0.12 $\log_{10}$ CFU/gm at harvest, 5.60 ± 0.12 $\log_{10}$ CFU/gm during transport and 5.56 ± 0.12 $\log_{10}$ CFU/gm at retail shop. The results are given in Table-2. Statistical analysis of comparison of mean *E.coli* counts at different stages of supply chain indicate non-significant differences

Table 2: Results of statistical analysis of Mean counts of *Staphylococcus aureus, E. coli* and Yeast & Molds from fish at various stages of supply chain

Stages	*Staphylococcus aureus* counts Log CFU/gm Mean ± SE	*E.coli* counts Log CFU/gm Mean ± SE	Mean Yeast and moulds counts (Log CFU/gm ± SE)
Harvest	5.36^{NS} ± 0.12	5.79^{NS} ± 0.12	0.00 ± 0.00
Transportation	5.71^{NS} ± 0.12	5.60^{NS} ± 0.12	5.41^{NS} ± 0.09
Retail Shop	5.80^{NS} ± 0.12	5.56^{NS} ± 0.12	5.74^{NS} ± 0.09

^{NS} – Non –significant at ($p<0.01$)

Table 3: Biochemical tests for *Vibrio* spp isolates

	Isolate Codes												
	Vi-1 (*)	Vi-2 (*)	Vi-3 (**)	Vi-4 (*)	Vi-5 (**)	Vi-6 (**)	Vi-7 (***)	Vi-8 (*)	Vi-9 (*)	Vi-10 (*)	Vi-11 (**)	Vi-12 (***)	Vi-13 (*)
Growth On TCBS	Y	Y		Y	G	G	G	Y	Y	Y	G	G	Y
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
0% NaCl	+	+	-	+	-	-	-	+	+	+	-	-	+
6% NaCl	-	-	+	-	+	+	+	-	-	-	+	+	-
8% NaCl	-	-	+	-	+	+	-	-	-	-	+	-	-
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	+	+	-	+	-	-	-	-	-	+	-	-	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	-	-	-	+	+	+	-	-	+

(*)- *Vibrio cholera*, (**)- *Vibrio parahamolyticus* and (***)- *Vibrio vulnificus*

in mean *E.coli* counts at various stages of supply chain. Yagoub (2009) evaluated hygienic quality and freshness of fresh fish in Khartoum fish market. The *E.coli* count found was in the range of 3×10^2 to 7×10^3 cfu/ml. *E.coli* counts of $1.4\pm0.10\times10^2$ cfu/gm to $4.8\pm0.45\times10^4$ cfu/gm were found in mola fish sold in Dhaka metropolish. Presence of *E.coli* in fish at various stages of supply chain is clearly indicative of contamination from environmental sources and handlers during supply chain.

The per cent positive-ness for *Salmonella* spp found was 16.66 per cent fish samples at harvest, 83.33 per cent during transport and 50 per cent at retail shop. The results are shown in Table 1. Similarly, *Salmonella* spp was found in 65.00 per cent in fish sold in retail market and 30 per cent in Super shop of Bangladesh (Begum *et al.*, 2010). It is evident interesting to observe that the percentage of *Salmonella* positive fish samples rose from 16.66 per cent at harvest to 83.33 per cent during transport and 50.00 per cent at retail shop. It is understood that the hygienic maintained during transportation is of low quality because of repeated use of gunny bags without hygienic interventions.

The all total 18 fish samples collected at different stages of supply chain were screened for *Clostridium* spp by using SPS agar. The percentages of positive samples were recorded. The results are depicted in Table 1. A total of 33.33 per cent samples were positive for *Clostridium* spp at harvest, followed by 50.00 per cent during transport and 100.00 per cent at retail shop. *Clostridia* are present in soil, water and intestinal tract of humans and other animals. Detection of *Clostridium* spp in fish samples is due to contamination from containers, handlers or dust. Because of spore forming ability organisms can withstand adverse environmental condition of drying, heating and certain

toxic compounds (Jay *et al*., 2005). In present study, it was found that right from harvest to retail shop the fish were exposed to various sources of *Clostridial* contamination like containers, handlers and dust thereby making them vulnerable to *Clostridial* infection. The results of present study are on similar lines as reported earlier (Shorbagy *et al*., 2012).

All 18 samples of fish at various stages of supply chain were screened on TCBS agar for primary isolation of *Vibrio* spp. A total of 13 *Vibrio* spp (72.22 per cent) isolates were obtained out of 18 samples based on colony characters on selective medium TCBS agar. It was observed that all samples were positive for *Vibrio* spp (100 per cent) at harvest. The percentages of isolations were reduced to 83.33 per cent during transportation and 33.33 per cent at retail shop. The results are given in Table-1. Pal and Das (2010) reported incidence of *Vibrio parahaemolyticus* in 66.66 per cent fish samples in Kolkata. Selective agar medium was used by Bakr *et al*. (2011) for isolation of *Vibrio* spp from seafood and recovery of 52 per cent *Vibrio* spp was reported. A total of 13 (72.22 per cent) *Vibrio* spp were isolated from 18 samples at various stages of supply chain. All isolated organisms were further differentiated according to their bio-chemical characteristics. Out of 13 *Vibrio* spp isolated 7 (38.88 per cent) were positive for *V. cholera*, 4 (22.22 per cent) were positive for *V. parahaemolyticus* and 2 (11.11 per cent) were positive for *V. vulnificus*. The results are shown in Tab-3. The presence of *Vibrio* spp in fish at harvest (100 per cent) may be due to their presence in reservoir environment which became a source of fish infection at harvest (Sudha *et al*., 2014). Presence of *Vibrio* spp at subsequent stages might be due to contamination from environment and handling.

The mean yeast and moulds counts were recorded from fish samples by using SDA agar at various stages of supply chain. It was observed that all 6 samples were negative for yeast and molds (0 per cent) at harvest. The percentages of isolations were increased to 50 per cent during transportation and 100 per cent at retail shop. The results are given in Table-1. The yeast and moulds found at various stages of supply chain were 0.00 ± 0.00 $\log_{10}$ CFU/gm at harvest, 5.41 ± 0.09 $\log_{10}$ CFU/gm during transport and 5.74 ± 0.09 $\log_{10}$ CFU/gm at retail shop. The mean did not differ significantly during various stages of supply chain. The results are depicted in table 2. Earlier Goja *et al*. (2013) found yeast and mould counts in the range 0.0-3.7×10^3 cfu/gm during microbial assessment of fresh fish sold in Ed Duenim, Sudan. Yeast and moulds affecting fish are opportunistic and may infect fish from environment and other sources of contamination. Temperature and moisture present at different stages of supply chain contributes to their growth and multiplication (Refai *et al*., 2010).

CONCLUSION

Microbial quality of freshwater fish (*Catla catla*) was found to be very good at harvest, transportation and retail shop in Parbhani city. Presence of organisms like *S. aureus*, *E.coli*, *Clostridium* spp, yeast and moluds at various stages of supply chain indicate presence of source of their contamination in the environment. Identification of *Salmonella* spp and *Vibrio* spp indicate concern from public health point of view.

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