Internet Journal of Food Safety, Vol.12, 2010, p.181-186

Internet Journal of Food Safety

The occurrence of *Escherichia coli* in fish samples isolated from different ponds of Nadia District, West Bengal, India

Chandraval Dutta [1,2*], Debasish Saha[2] , Ashis Kumar Panigrahi[1] and Chandan Sengupta[2]

1 Aquaculture Extension Laboratory, Department of Zoology, University of Kalyani, Nadia, West Bengal, India
2 Microbiology Laboratory, Department of Botany, University of Kalyani, Nadia, West Bengal, India

Abstract

The prevalence of *Escherichia coli* in fish and water samples collected from Nadia District of West Bengal in India were studied. Fresh and ice preserved *Labeo rohita, Catla catla, Cirrhinus mrigala, Labeo bata* were examined for total bacterial load and presence of *E.coli* by standard microbiological techniques. The number of bacterial flora is more or less similar in all fish samples collected from four different ponds of Nadia District. *E.coli* loads remained higher in *Labeo rohita* (rohu) fish than other three fishes. The fishes were contaminated with faecal coliforms like *E.coli* indicating poor hygiene and sanitary condition.

Key words: prevalence, *E.coli*, faecal coliform, fish, Nadia,

Introduction

Food borne infection in relation to public health has been considered with great importance in the whole world. The majority of foods borne diseases are linked to foods of animals, including fish and shell fish origin. According to Gram and Huss 2000 the incidents of food borne illness from fish and shellfish products are higher in Asian Countries like Japan, Korea, and India than in North America and Europe.

In West Bengal fish is a staple food item for Bengalis irrespective of caste and creed. The incidents of food borne illness are very frequent in diarrhea prone state like West Bengal. A numerous group of bacteria particularly coliform group enters in to the fish gut through water, sediment and food. Faecal coliform such as *Escherichia coli* is a common part of normal flora of human intestine, warm blooded animals, birds and many animals.

* Corresponding author. mailing address Department of Zoology, University of Kalyani, Kalyani , Nadia -741235 West Bengal, India
Email: chandraval@gmail.com

The occurrence of faecal coliform in fish represents the pollution level of pond environment because coliforms are not normal flora in fish. (Cohen, 1973). *E.coli* is frequent contaminants of food and water and a well recognized food borne pathogen. Among these most are harmless but few strains are responsible for causing diarrhea (ICMSF 2002). The contamination of food of fish origin with pathogenic *E.coli* probably occurs during handling of fish (Ayulo et al. 1994, Asai et al. 1999). Thampuran et al. (2005) reported that usually associated with different disease, the versatility of *E.coli* strains due to the fact that different strains have acquired different set of virulence genes (Teophilo et al.2002.)An outbreak of diarrhoeal illness causes by ingestion of food contaminated with enterotoxigenic *E.coli* the microbial quality of tissue samples of tilapia fish were contaminated with fecal coliform mostly *Escherichia coli*. The presence of *E.coli* is also reported from finfish as well as shellfish in different markets around Kolkata, West Bengal (Manna et al. 2008).Different strains of *E.coli* are

was reported in Japan.(Mitsuda et al.1998).Brazillian authors have isolated 18 toxigenic *E.coli* from markets in Brazil.(Vieira et al.2001).

The objectives of this study were to assess the microbiological quality of fish and water collected from different season and to survey the prevalence of *E.coli* in fish and water samples from the above stated sources.

Material and Methods

Fishes were collected from four different ponds located in different places of Nadia district. All fish samples were collected in drawn in sterile 500 ml bottles from different sites of each pond. Fish samples brought to the laboratory, maintaining temperature at 4^0C, kept in ice. The study was conducted during February to April, 2010.Fish tissues including skin were cut and homogenized in normal saline (0.85 NaCl) suspension. Then homogenized tissue samples were serially diluted in normal saline and plated on to nutrient agar (Himedia Lab, Mumbai, India) for total plate count(TPC) of bacteria and incubated at 37^0Cfor 24 hours.For isolation of *E.coli* fish and water samples were enriched in *E coli* broth(EC broth) at 44^0 C for 24 hours. Then the enriched EC medium was streaked on to eosin methylene blue agar (EMB, Himedia) and incubated at at 37^0Cfor 24 hours. Then the plates were examined for characteristic metallic sheen which were further purified and identified as *E. coli.* The presumptive *E.coli* isolates were identified by performing following tests: gram staining reaction, oxidation reaction, sugar (lactose, mannitol and cellobiose) utilization and IMViC reaction.

Results

In the study the microbial load of the fish surface as well as the pond water sample were estimated at one month time intervals. The results are shown in Table -1. It is apparent from the results that the number of bacterial flora more or less same in all fish samples collected from four different ponds of Nadia District. The number of bacteria slightly high in fishes collected from Kalyani pond whereas it is comparatively less in Santipur pond. This is may be due to the effect of some physical factors of pond ambient environment. The result further showed that the quantity of bacteria gradually increased due to course of time. . In a separate study the microbial load of water samples were measured (Table -2). It was evident from the study that water samples possess huge no of bacteria. Here also microbial load was high in Kalyani water than other test water samples. In the study the number of *E.coli* from four different commonly used fish samples of west Bengal was estimated. The results are shown in (Table 3).

Table -1: Microbial load of fish body surface obtained in various time period in different ponds of Nadia district.

Time of collection[a]	Number of bacterial colony[b] on fish surface in different ponds of Nadia district ($x10^5$)			
	Kalyani	Krishnanagar	Ranaghat	Santipur
February(2010)	0.47(20)	0.34(21)	0.24(20)	0.14(19)
March(2010)	3.6(23)[c]	2.80(26)	1.8(24)	1.2(25)
April(2010)	56(30)	43(34)	32(31)	10(31)

[a] Fish tissue of bacterial colony obtained per gm of tissue was enumerated by dilution plate count technique on nutrient agar medium.

[b] Fish tissue sample was collected from different pond. It indicates periods in month.

[c] Figure in the parenthesis indicated the ambient temperature of the pond water.

Table -2 Microbial load of pond water obtained in various time period in different ponds of Nadia district.

Time of collection[a]	Number of bacterial colony[b] on fish surface in different ponds of Nadia district ($x10^5$)			
	Kalyani	Krishnanagar	Ranaghat	Santipur
February(2010)	0.92(20)	0.66(21)	0.49(20)	0.38(19)
March(2010)	7.5(23)[c]	5.2(26)	3.7(24)	5.1(25)
April(2010)	125(30)	86(34)	75(31)	67.5(31)

[a] Water samples were collected aseptically in sterile tube from different ponds at indicated period.

[b] Number of bacterial colony obtained per ml. of water enumerated by dilution plate technique on nutrient agar.

[c] Figure in the parenthesis indicated the ambient temperature of the pond water.

Table-3: Prevalence of *E.coli* in different fish samples obtained in ponds of Nadia District

Fish[a]	Number of [b] Sample tested	Number of [c] sample Possess *E.coli* (x 10^5)	Percentage[d] of *E.coli* prevalence
Labeo rohita	12	10	83.33%
Catla catla	7	5	71.42%
Cirrhinus mrigala	3	2	66.0%
Labeo bata	10	7	70.0%
	Total -32[e]	24(75%)[f]	

[a] Fish samples were collected from local ponds.

[b.] Number of fish samples tested for harboring *E.coli*

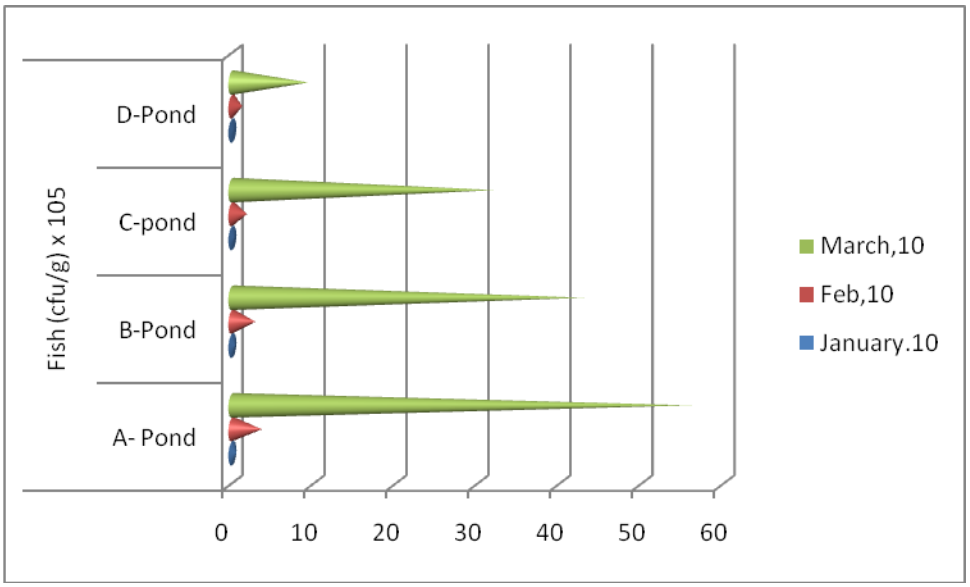
[c.] Number of samples gave positive response of *E.coli* existence.

[d.] Percentage of fish possesses *E.coli.*

[e]. Total number of fish samples tested.

[f.] Percentage of *E.coli* obtained in test samples.

A. Fish body (cfu/g)x10^5



B. Pond water (cfu/g)x10^5

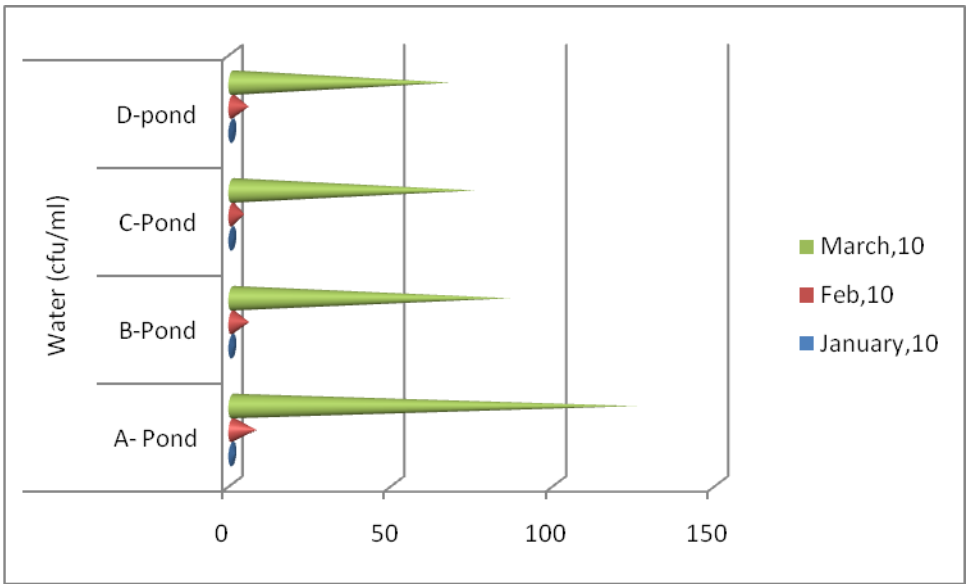


Fig:1 Comparison of *E.coli* load in various time gap (month) in per unit weight of Fish (A) and per unit amount of watewr (B) sample.

Discussion

In the sudy the prevalence of *E.coli* load on fish body was estimated specifically on rohu fish which is extensively used by bengali fish lover following standard dilution plate count techniques. Simultaneously the existence of *E.coli* in pond water was also ascertained to verify the ocurrence of the bacterium in the ecosystem of fish dweller.

E.coli has been traditionally recognized as an indicator organism of faecal contamination of water and seafood (Geldreich, 1997). Testing of seafood for the presence of *E. coli* is still a gold standard used to assess the faecal contamination in seafood processing plants in India and elsewhere. *E.coli* is a normal inhabitant of the intestinal tracts of all warm blooded animals. However, strains of human pathogenic *E. coli* have evolved that are recorded as causative agents of a broad range of human diseases compared to any other pathogenic bacteria (Nataro & Kaper 1998; Paton & Paton 1998).

The results obtained in this study indicated that a considerable number of *E.coli* existed on the body surface of the fish. The results therefore in confirmation of the previous records that *E.coli* is one of the most important prevalent microorganisms on fish body (Levine, 1987). Moreover the results further showed that the number of *E.coli* is more or less same in all the fish samples collected from different sources. The result therefore in confirmation of the previous records that the number of the existing microorganisms of a particular ecosystem is mainly depends on various physicochemical factors of the surrounding area (Alexander, 1971).

As the fishes were collected from different water body of Nadia District and the places, which were adjoining to each other so the surrounding physiochemical factors of the ponds which regulate the quantity and quality of microorganisms are more or less same which reflect the microbial load assessment. The results again confirm the idea that the roles of abiotic environment factors have profound influence on the quantity and distribution of micro organisms (Alexander, 1971).

Another important finding of this study is that the quantity of *E.coli* had increased remarkably at the months of April than February and March. This is due to the increased temperature effects on the population dynamics of *E.coli*. It was of interest to note that in April the pond water temperature became 31^0C to 34^0C which is within the range of incubation temperature of *E.coli*. As the temperature is extremely suitable for *E.coli* growth and proliferation, so the numbers of the bacterium was increased.

Furthermore, the temperature remain between 30^0C to 40^0C particularly in hot summer and as during the period the *E.coli* population becomes high so special caution measurement should taken for this alarming condition of prevalence of *E.coli* load in fish body and pond water also. As *E.coli* is highly pathogenic and cause diarrhoea (Levine, 1987). So, special care and further investigation are required to tackle this situation. Moreover, it is well evidence that *E.coli* causes food poisoning and food spoilage (Armstrong et al.1996).

In a separate study it was noted that as compare to three other fishes like catla, mrigala and bata the percentage of *E.coli* load and other organisms remained high in rohu fish which also gave an indication to take special care about rohu fish.

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