World Journal of Fish and Marine Sciences 1 (3): 160-166, 2009
ISSN 1992-0083

Coliform Bacteria in Nile Tilapia, *Oreochromis niloticus* of Shrimp-*Gher*, Pond and Fish Market

[1]*Shankar Chandra Mandal,* [1]*Mahmud Hasan,* [1]*Mohammad Shamsur Rahman,*
[1]*Mozaffor Hossain Manik,* [2]*Zahid Hayat Mahmud and* [2]*M.D. Sirajul Islam*

[1]Department of Fisheries, University of Dhaka, Dhaka 1000, Bangladesh
[2]International Centre for Diarrhoeal Disease Research, G.P.O. Box 128, Dhaka 1000, Bangladesh

Abstract: This study investigated the abundance of coliform bacteria in Nile tilapia sampled from different sources. Densities of total aerobic bacteria, total coliform (TC), faecal coliform, (FC) and *Escherichia coli* were measured from different organs of Nile tilapia sampled from pond, *gher* and market using serial dilution and spread plate techniques. Significant differences were observed in various parameters of bacterial density between and within different organs of Nile tilapia ($p<0.05$). Significantly higher density of faecal coliform was detected in the muscle, gill and intestine in Nile tilapia sampled from pond than that of market. The highest density of total aerobic bacteria ($8.81 \pm 0.45 \times 10^5$ cfu/g), TC ($3.00 \pm 0.20 \times 10^4$ cfu/g) and *Escherichia coli* ($1.45 \pm 0.19 \times 10^3$ cfu/g) were measured in the intestine and FC ($3.00 \pm 0.67 \times 10^3$ cfu/g) in the gill of Nile tilapia sampled from market. Findings of the present study suggest that Nile tilapia may be faecally contaminated during culture period, storage and transportation and unhygienic marketing.

Key words: Nile tilapia % Coliform % Gher % Satkhira % Chandpur % Bangladesh

INTRODUCTION

Nile tilapia is an alien fish introduced in Bangladesh in 1974 from Thailand [1]. Because of their faster growth rate, tolerance to harsh environment and ease culture technique, tilapia offers the possibility of commercial and home-grown protein sources where wild capture fisheries are being depleted. Tilapia is more suitable for culture in the shallow and small ponds. Nile tilapia can tolerate, grow and even reproduce in saline waters with an optimum level 10-20 ‰ [2]. Tilapia also grows well in brackish water attaining 200-350 g in 4 to 6 months [3]. Nile tilapia has potential in Bangladesh as an alternative and additional species among farmed fishes [4].

Most of the tilapia production in Bangladesh comes from aquaculture which largely depends to a greater extent on aquatic environment. Water quality is the main factor that determines the degree of production. Contaminated water is not suitable for aquaculture. Contamination may result from rupturing fish intestine during poor processing or inadequate washing.

It has been suggested that intestinal microflora is the causative agent for food spoilage [5]. Contamination of fish from enteric bacteria of human or animal origin may also be responsible for various food spoilages [6]. Fish take a large number of bacteria into their gut from water, sediment and food [7]. In a previous study it has been demonstrated that the bacterial flora in fish reflects the aquatic environments [8]. It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria, particularly the coliform group [9-11]. Faecal coliforms such as *Escherichia coli* usually originate from faeces of warm blooded animals. Faecal coliform in fish demonstrates the level of pollution of their environment because coliforns are not the normal flora of bacteria in fish [12]. The enteric bacilli include *E. coli*, *Klebsiella* spp. *Citrobacte*r spp. *Enterobacter* spp., *Serratia* spp. and *Edwarsiella* spp. [12]. However, bacterial density in Nile tilapia sampled from pond, *gher* and market has never been reported. Thus the present study was designed to investigate the occurrence of viable coliforms quantitatively in different organs of Nile tilapia reared in ponds, *ghers* and waters of the same.

Corresponding Author: Dr. Mahmud Hasan, Associate Professor, Department of Fisheries, University of Dhaka, Dhaka 1000, Bangladesh

MATERIALS AND METHODS

Nile tilapia (28.72±5.35 cm; 519.38±259.10 g, mean±SEM) was drawn from four different *ghers* in Satkhira, a South-western district of Bangladesh which is nearly 483 km away from the capital city Dhaka and pond in Chandpur of central Bangladesh. The ghers and ponds were at different locations with variable physical properties (Table 1a and b). Tilapia was also sampled from Kachukhet and Farm gate fish markets of Dhaka metropolis. Samples from muscle, gill and intestine were separately examined for each specimen. Geographical location was determined by using a GPS meter (GPS 12, GARMIN Olathe, KS, USA).

Collection of Fish and Water Samples: Tilapia was collected from four different *ghers* and ponds. Water samples were drawn in sterile 500 mL bottles from two different sites of each *gher* and pond and brought to the laboratory in icebox maintaining temperature at 4°C [13]. Both fish and water were transported directly to the Environmental Microbiology Laboratory of ICDDR,B in an insulated box filled with cool packs (Johnny Plastic Ice; Pelton Sheperd, Stockton, CA, USA) within 6-8 hours of sampling at 4°C. Fish were caught by Ghuni, a local fishing gear and by cast net. The study was conducted during April to September 2008. Sampling was drawn between 1:30 and 4:00 pm.

Processing of Fish Samples: Samples were processed for bacteriological analyses within 8-12 hours of sampling following aseptic techniques. Market fish were analyzed within 2-4 hours of collection.

Total Aerobic Bacteria: Luria agar (a nutrient agar) was used to prepare the culture medium. A sub-sample of 1 g was taken from homogenized tissue of each sample and mixed with 9 mL sterile PBS solution to prepare a 10^{-1} dilution. Then, subsequent serial dilutions were prepared from 10^{-2} to 10^{-5}. The spread plate method was used to enumerate the total bacterial density [14]. A sub-sample of 100 µL samples from each dilution with three replicates was used to count bacteria as colony forming unit (CFU) per g of sampled fish. Bacterial density as cfu/g for 3 replicates were initially averaged and used for final calculation. All equipment and chemicals used were sterilized properly prior to use.

Table 1(a): Location and physical criteria of the *ghers*

Gher	Latitude	Longitude	Area (ha)	Water depth (ft)
1	22°40.264' N	89°8.735' E	2.5	3-5
2	22°40.239' N	89°8.731' E	2.0	2-4
3	22°40.241' N	89°8.725' E	4.0	3-5
4	22°40.251' N	89°8.740' E	6.5	4-5

(b): Location and physical criteria of the ponds

Pond	Latitude	Longitude	Area (ha)	Water depth (ft)
1	23°19.715' N	90°40.720' E	1.0	4-5
2	23°20.787' N	90°40.161' E	1.0	3-4
3	23°20.875' N	90°41.616' E	1.5	3-5
4	23°20.890' N	89°8.840' E	1.5	4-5

TC: The detect the total coliform; 100 µl samples of serially diluted solutions were spreaded on the mFC plate and incubated at 37°C for 18 to 24 hours. Blue colonies were considered as total coliform.

FC: Similar procedure to TC was followed to enumerate the fecal coliform. However, plates were incubated at 44°C for 18 to 24 hours.

E. Coli: For the enumeration of *E. coli*, 100µl samples of serially diluted solutions were spreaded on the McConkey agar plates and incubated at 44°C for 18 to 24 hours. Pink colonies were counted as *E. coli*.

Processing of Water Samples: Each water sample was analyzed to determine total aerobic bacteria, TC, FC and *E coli* following procedures described [13, 15]. In brief, for the enumeration of TBC in water, 100 µL sample water was mixed with 900 µL Normal saline (0.85% NaCl solution) to prepare 10^{-1} dilution. Then 100 µL of diluted water samples were dropped on Lurria Agar plates. The plates were then incubated at 37°C for 18-24h. For TC and FC and *E. coli*, 100 mL of water samples were filtered through 0.22 µm pore-size membrane filter (Milipore corp., Bedford, MA, USA) and filters were placed on membrane faecal coliforms (mFC) agar plates for TC and FC and on McConkey agar plates for *E. coli*. The mFC plates were incubated at 37 and 44°C for 18-24 h for TC and FC respectively. McConkey agar plates were incubated at 44°C for 18-24h.

The characteristic blue colonies on mFC plates were counted as TC and FC and expressed as colony forming units (CFU) per 100 mL. Pink colonies on McConkey agar plates were counted as *E. coli* and expressed as CFU per 100 mL of water [13].

In Situ Measurement of Physicochemical Parameters in Water Samples: At the site of the *gher* and pond water P^H, salinity, TDS (Total Suspended Solids), water temperature, DO (Dissolved Oxygen), conductivity were measured using a portable Conductivity meter (HACH, CO150 conductivity meter, USA). Dissolved oxygen and pH were measured using a portable (HACH DO175, USA) DO meter and Orion field pH meter (210A, Orion Laboratories, USA) respectively.

Statistical Analysis: Bacterial density data were transformed into natural log before statistical analysis. The means of bacterial load were compared by using ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical software SPSS version 11.5 was used to analyze the data with the level of significance at $p<0.05$.

RESULTS AND DISCUSSION

Physiochemical Parameters of *Gher* and Pond Water: Water quality variables of *gher* water were found suitable for the culture of Nile tilapia (Table 2) [16]. The salinity of pond water was near to zero. DO and pH of pond water were within tolerance limit for Nile tilapia (Table 2). Nile tilapia can survive 3–25‰ salinity [16]. Therefore, *gher* water was suitable for better growth of Nile tilapia. However, all water quality variables of the pond and *gher* water were suitable for bacterial proliferation particularly the water temperature was very much suitable for the coliform bacteria. The observed high bacterial count detected in fish and water samples taken from both pond and *gher* could be because of that.

Bacterial Loads in Nile Tilapia Sampled from *Gher* and Market

Densities (cfu/g) of Total Aerobic Bacteria: The density of total aerobic bacteria found in the muscle, gill and intestine of Nile tilapia sampled from *gher* were indifferent (Figure1). Similarly, the total bacterial count measured in the gill and intestine of the same fish sampled from market was similar but significantly higher than that of muscle.

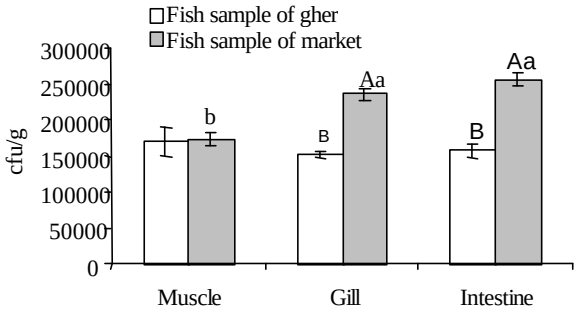

Fig. 1: Density (cfu/g) of total aerobic bacteria in the muscle, gill and intestine of Nile tilapia reared in *gher* sampled form *gher* and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

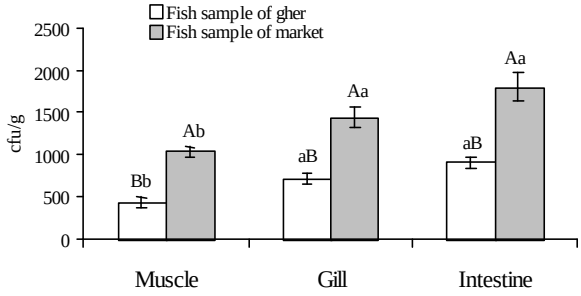

Fig. 2: Density (cfu/g) of TC in muscle, gill and intestine of Nile tilapia reared in *gher* sampled form *gher* and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

In muscle, the bacterial build up observed in the samples sampled from *gher* and market was similar, while total bacterial density found in the gill and intestine of the same fish sampled from market were significantly higher than those of *gher*.

The higher density of total aerobic bacteria found in gill and intestine of Nile tilapia sampled from market might be due to quick proliferation after catching and during transportation and storage.

Table 2: Physiochemical parameters (means ± SEM) of sampled *gher* and pond water

	Water quality variables					
System	DO (mg/L)	Water Temp (°C)	P^H	Salinity (‰)	TDS (mg/L)	Conductivity (µs/cm)
Gher	7.58±0.34	33.10±0.27	7.99±0.89	5.51±0.17	5263.75±145.27	9923.75±629.45
Pond	6.09±0.22	30.03±0.29	6.44±0.55	0.05±0.02	59.89±8.37	122.34±17.01

Preservation in low quality ice, handling with contaminated hands could also be responsible for this higher density of aerobic bacteria.

Densities (cfu/g) of TC: The concentration of total coliform found in the gill and intestine of Nile tilapia harvested from *gher* was similar but significantly higher than that of muscle (Figure 2). Similarly, the density of TC detected in the gill and intestine of the same fish sampled from market was also similar and significantly higher than that of muscle. However, the densities of TC detected in all the three organs in Nile tilapia taken from market were significantly higher than that of *gher*.

Densities (cfu/g) of FC: The densities of fecal coliform observed in the gill and intestine of tilapia collected from *gher* were not different but significantly higher to that of muscle (Figure 3). Similarly, the count of FC detected in the gill and intestine of the same fish sampled from market was also similar and significantly higher than that of muscle. On the other hand, the densities of FC found in all the three organs in tilapia collected from market were significantly higher to that of *gher*.

Densities (cfu/g) of E. Coli: The concentration of *E. coli* detected in the gill and intestine of tilapia harvested from *gher* was similar but significantly higher than that of muscle (Figure 4). The load of that bacterium found in all the three organs of the same fish sampled from market was similar. On the other hand, the densities of *E. coli* found in all the three organs in tilapia gained from market were significantly higher than that of *gher*.

In all the cases the bacterial loads in different organs of Nile sampled from market were significantly higher than that of *gher* ($p<0.05$). The higher density may be due to lack of proper hygiene of fish market. Culture environment mainly the water quality may enhance the growth of bacteria. Improper handling and processing may cause the higher density ob bacteria, especially the coliform bacteria in fish sampled from market. Cross contamination may be another reason.

Bacterial Loads in Nile Tilapia Sampled from Pond and Market

Densities (cfu/g) of Total Aerobic Bacteria: The densities of total aerobic bacteria found in the muscle, gill and intestine of Nile tilapia collected from pond were similar (Figure 5). In the same way, the total bacterial count observed in the same organs of the same fish sampled from market was also similar. However, the total bacterial

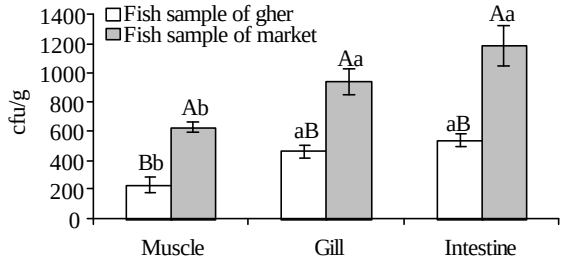

Fig. 3: Density (cfu/g) of FC in muscle, gill and intestine of Nile tilapia reared in *gher* sampled from *gher* and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

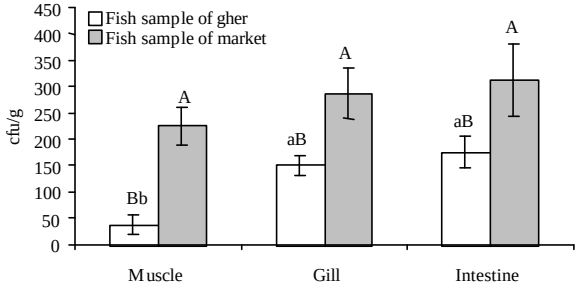

Fig. 4: Density (cfu/g) of E. coli in muscle, gill and intestine of Nile tilapia reared in *gher* sampled from *gher* and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

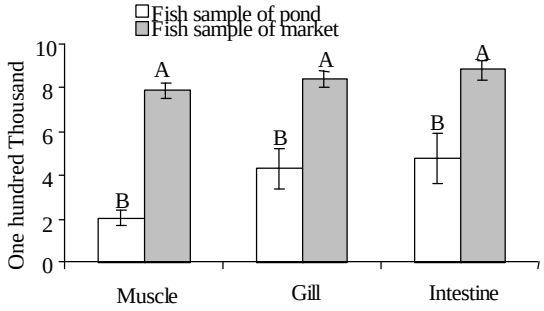

Fig. 5: Density (cfu/g) of Total Aerobic Bacteria found in the muscle, gill and intestine of Nile tilapia reared in pond sampled from pond and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

count found in all the three organs of tilapia sampled from market was significantly higher than that of pond. Similar result was observed earlier in freshwater catfish [17].

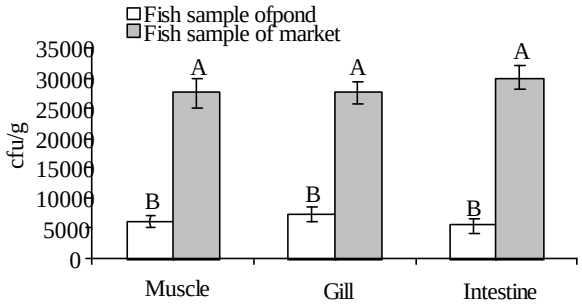

Fig. 6: Density (cfu/g) of TC in muscle, gill and intestine of Nile tilapia reared in pond sampled from pond and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

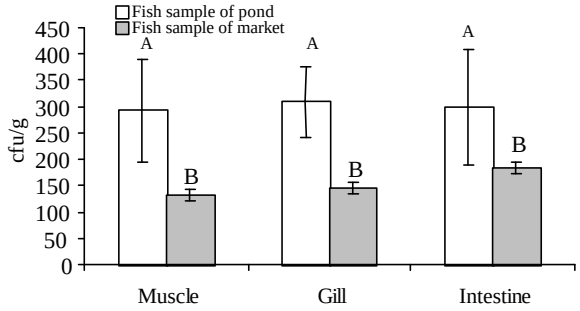

Fig. 7: Density (cfu/g) of FC in muscle, gill and intestine of Nile tilapia reared in pond sampled from pond and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

Densities (cfu/g) of TC: The blooms of total coliform observed in the muscle, gill and intestine of Nile tilapia collected from pond were similar (Figure 6). Likewise, the density of total coliform detected in the same organs of the same fish collected from market was also similar. However, the total coliform found in all the three organs of Nile tilapia sampled from market was significantly higher than that of pond.

Densities (cfu/g) of FC: The concentration of fecal coliform found in the muscle, gill and intestine of Nile tilapia harvested from pond was indifferent (Figure 7). Similarly, the blooms of FC detected in the same organs of the same fish collected from market were also indifferent. On the other hand, the FC observed in all the three organs of Nile tilapia sampled from pond was significantly higher than in the market ($p<0.05$). This could be due to highly faecal contamination of source water.

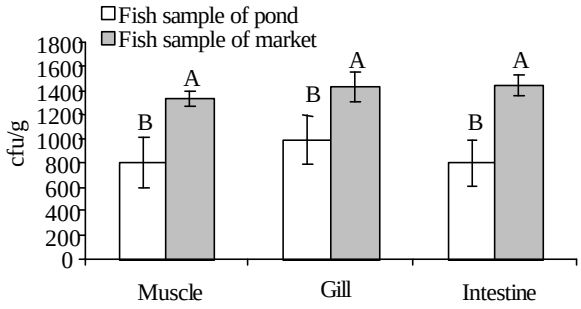

Fig. 8: Density (cfu/g) E. coli in muscle, gill and intestine of Nile tilapia reared in pond sampled from pond and market. Bars (mean ± SEM) with same color different letters are significantly different. Capital letters denote significant differences within organ ($p<0.05$).

Table 3: Densities (cfu/mL water) of different bacteria in water sampled from gher and pond

	Parameters			
System	TBC	TC	FC	*E. coli*
Gher	$2.55\pm0.15\times10^{3}$	$1.55\pm0.12\times10^{1}$	$9.50\pm1.09\times10^{0}$	$2.74\pm0.38\times10^{0}$
Pond	$3.56\pm0.53\times10^{3}$	$1.95\pm0.64\times10^{2}$	$1.73\pm0.18\times10^{1}$	$3.32\pm0.91\times10^{0}$

Densities (cfu/g) of E. Coli: The densities of *E. coli* found in the muscle, gill and intestine of Nile tilapia collected from pond was similar (Figure 8). Similarly, the load FC detected in the same organs of the same fish collected from market was also similar. On the other hand, the concentration of FC observed in all the three organs of tilapia collected from market was significantly higher than that of pond.

Bacterial Load in Water Sampled from Gher and Pond: Total aerobic bacteria detected in both gher and pond water was beyond the lower limit suitable for fish culture (Table 3). Load of TC, FC and *E. coli* were also high.

A study reported total aerobic bacteria as 2.1×10^{3} to 7.1×10^{5} and 2.3×10^{3} to 4.1×10^{6} cfu/mL of water samples taken from two different fish ponds [18] that is in agreement with the present study. A previous study has demonstrated 6.0×10^{2} to 1.0×10^{4} cfu/mL heterotrophic bacterial load in brackish that is also similar to the findings of the current study [19].

A level of coliform of = 10 per mL of water is suitable for pond aquaculture [20]. The loads of different bacteria observed in water samples taken from pond and gher were higher than the recommended value for fish culture. The higher density in fish may be due to their consumption of bacteria for long time through food and water. Another

reason may be that the high water temperature in the pond (30.03 ± 0.29°C) and gher (33.10 ± 0.27°C) water was close to optimum for many mesophilic bacteria in natural system [21].The highest load of total aerobic bacteria (8.81 ± 0.45 × 10^5 cfu/g), TC (3.00 ± 0.20×10^4 cfu/g) and *E. coli* (1.45 ± 0.19×10^3 cfu/g) were detected in the intestine of Nile tilapia reared in pond sampled from market. But the highest density of FC (3.00 ± 0.67×10^3 cfu/g) was found in the gill of the same fish. The presence of high bacterial load in intestine and gill of Nile tilapia might be due to high metabolic activity of fish associated with increased feeding rates at higher [22]. A study observed similar bacterial load in the intestine of tilapia [23]. Bacterial load in the intestine of freshwater tilapia in Saudi Arabia was reported as 6.8×10^6 to 7.5×10^7cfu/g [24] which is in comparable to the present study.

This higher density of coliform bacteria in water, especially the faecal coliform, is responsible for higher density of these bacteria in fish body. Quick spoilage of fish after catching might be due to this higher density of these bacteria. *E. coli* are human originated bacteria which may be responsible for different enteric disease in human body. The higher density in fish body may be due to secondary contamination during handling and storage. Large quantities of coliform bacteria in water and fish are not pathogenic to human, but may indicate a higher risk of pathogens being present [25]. Dysentery, typhoid fever, bacterial gastroenteritis and many other water borne disease may coincide with faecal coliform contamination. The presence of faecal coliform may affect humans more than it does aquatic organisms [25].

Fishes are very much susceptible to contamination with different bacteria because of their highly perishable protein content in their body. Coliforms are not the normal flora of bacteria in fish. Due to deposition of human excreta in pond, water is contaminated and when this contaminated water is ingested by the fish, they become contaminated. In the present study the fish samples of different sources were contaminated with total aerobic bacteria as well as total coliform, fecal coliform and *E. coli*. This bacterial population was higher in Nile tilapia sampled from market except the faecal coliform which was higher in fish sampled from pond. This might be due to the contamination of source water from where the fishes were caught or might be due to secondary contamination during the time of handling as well as storage of fishes in ice made with contaminated water.

In Bangladesh every day 20,000 metric tons of human excreta are deposited on public lands and waterways and is one of the main causes of contamination of surface water [26]. During the time of rainy season the fecal matters of various sources are washed away from the contaminated land and are ultimately carried into different water bodies. Moreover, due to the poor sanitary condition of the country most of the latrines in rural settings are directly connected to the pond and other water bodies. Pets, especially dogs, can contribute to faecal contamination of surface waters. Runoff from roads, parking lots and yards can carry animal wastes to pond and ghers through storm sewers. Birds can be a significant source of faecal coliform bacteria. Swans, geese and other waterfowl can all elevate bacterial counts in ponds and ghers [25]. Thus, virtually all the aquatic habitats of Bangladesh are heavily contaminated with fecal coliform bacteria. Fishes, which live in these polluted habitats thus can easily intake these bacteria during feeding along with contaminated aquatic foods.

According to International Commission on the Microbiological Specification of Foods [27] acceptable limit of total bacterial counts, total coliform and faecal coliform for white fish is 5×10^5, 10^2 and 10 cfu per gram respectively and *E. coli should* not be present. Therefore, the bacterial loads found in this study for Nile tilapia was beyond the standard value, which indicate their unacceptability as food from public health point of view.

Fish of good quality should have counts of total bacteria of less than 10^5 per gram and faecal coliforms and total coliforms should not exceed 10/gm and 100/gm respectively [28]. Total coliform, faecal coliform and *E. coli* count of Nile tilapia of different sources examined in this study exceeded the acceptable limit recommended by Food and Agricultural organization [28]. This indicates human health risk due to consumption of tilapia collected from pond and gher. Therefore, precautions should be taken to prevent contamination during harvesting as well as post harvest handling of fishes. Depending on the sources and other environmental factors, a wide range of variation in distribution of microflora in fish has been reported [29]. The present study correlate with this finding and hence showed variation of bacterial count in tilapia of different sources. Therefore, precaution should me taken to prevent contamination during harvesting as well as post harvest handling of fishes. Water quality for aquaculture purpose should be maintained.

REFERENCES

1. Rahman, A.A.K., 1985. Introduction of Exotic Fishes in Bangladesh. Fisheries Research and Training Centre, (booklet). DOF. Chandpur, Bangladesh, pp: 15.
2. Suresh, A.V. and C. Kwei Lin, 1992. Tilapia culture in saline waters: a review. Aquaculture, 106: 201-226.

3. Romana-Eguia, M.R.R. and R.V. Eguia, 1999. Growth of five Asian red tilapia strains in saline environments. Aquaculture, 173: 161-170.
4. Prunet, P. and M. Bornancin, 1989. Physiology of salinity tolerance in tilapia: an update of basic and applied aspects, Aquat. Living Resour., 2: 91-97.
5. Kaneko, S., 1971. Microbiological study of fresh fish. New Food Industry, 13: 76-80.
6. Geldreich, E.E. and N.A. Clarke, 1966. Bacterial pollution indicators in the intestinal tract of freshwater fish. Applied Microbiol., 14: 429-437.
7. Sugita, H., M. Tsunohara, T. Ohkoshi and Y. Deguchi, 1988. The establishment of an intestinal microflora in developing goldfish (Carassius *auratus*) of culture ponds. Microbiol Ecol., 15: 333-344.
8. Shewan, J.M. and G. Hopps, 1967. The bacteriology of fish spoilage and preservation. Progress in Industries Microbiology (ed. By D.J.D. Hockenhull). Illfe Books, London.
9. Leung, C., Y. Huang and O. Pancorbo, 1990. Bacterial flora associated with a Nigerian freshwater fish culture. Journal of Aquaculture in the Tropics, 5: 87-90.
10. Pulella, S., C. Fernandes, G.J. Flick, G.S. Libey, S.A. Smith and C.W. Coale, 1998. Indicative and pathogenic microbiological quality of aquacultured finfish grown in different production systems. Journal of Food Protection, 61: 205-210.
11. Ramos, M. and W.J. Lyon, 2000. Reduction of endogenous bacteria associated with catfish fillets using the grovac process. Journal of Food Protection, 63: 1231-1239.
12. Cohen, J. and H.I. Shuval, 1973. Coliform, fecal coliform and fecal streptococci as indicators of water pollution. Water Soil Pollution, 2: 85-95.
13. American Public Health Association, 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA, Washington, D.C.
14. Hasan, M. and A.N. Bart, 2007. Improved survival of rohu, *Labeo rohita* (Hamilton-Buchanan) and silver carp, *Hypophthalmichthys molitrix* (Valenciennes) fingerlings using low-dose quinaldine and benzocaine during transport. Aquaculture Res., 38: 50-58.
15. Islam, M.S., A. Huq, A. Siddika,M.N.H. Khan, M.M. Golder, M.A. Siddique, A.N.M.H. Kabir and R.R. Colwell, 2001. Microbiological analysis of tube-well water in a rural area of Bangladesh. Applied environ. Microbiol, 67: 3328-3330.
16. Hussain, M.G., 2004. Farming of Tilapia: Breeding Plans, Mass Seed Production and Aquaculture Techniques. Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh, pp: 10.
17. Mobin, S.M.A., M.B.R. Chowdhury, M.S. Islam and M.N. Uddin, 2001. Status of bacterial flora in the intestine of two freshwater catfish. Bangladesh J. Life Sci., 13(1and2): 149-155.
18. Nahiduzzaman, M., M.A. Ahsan, M.B.R. Chowdhury and M.A.R. Mridha, 2000. Status on Bacterial Flora in a Farmed Catfish, *Clarias* Hybrid. Pakistan Journal of Biological Sci., 3(3): 429-432.
19. Maugeri, T., D. Caccamo and D. Gugliandolo, 2000. Potentially pathogenic vibrios in brackish waters and mussels. J. Appl. Microbiol., 89: 261-266.
20. World Health Organization, 1989. Health Guidelines for the use of Wastewater in Agriculture and Aquaculture. WHO Technical Report Series, Number 778. World Health Organization, Geneva, Switzerland.
21. Rheinheimer, G., 1985. Aquatic microbiology, 3rd ed. University of Kiel, West Germany. John Willy and Sons, Chichester, New York, Brisbane, Toronto., pp: 257.
22. Al-Harbi, A.H. and M.N. Uddin, 2004. Seasonal variation in the intestinal flora of hybrid tilapia (Oreochromis *niloticus* × *Oreochromis aureus*) cultured in earthen pond in Saudi Arabia. Aquaculture, 229: 37-44.
23. Chowdhury, M.B.R., M. Muniruzzaman and M.N. Uddin, 1989. Studies on the bacterial flora of tilapia *Oerochromis niloticus.* Bangladesh Journal of Aquaculture, 11: 65-70.
24. Al-Harbi, A.H. and M.N. Uddin, 2005. Bacterial diversity of tilapia (Oreochromis *niloticus* × *Oreochromis aureus*) cultured in brackish water in Saudi Arabia. Aquaculture, 250: 566-572.
25. Doyle, M.P. and M.C. Ericson, 2006. Clossing the door on the faecal coliform assay. Microbe., 1: 162-163.
26. GOB, 2003. Water sanitation and hygiene promotion, a national strategy for economic growth, poverty reduction and social development. Presented to the GED, planning commission, 05th March 2003. Government of Bangladesh.
27. International Commission on the Microbiological Specification of Foods (ICMSF), 1982. Microorganisms in food. Vol. 2, Sampling for microbiological analysis: principles and specific applications. Univ. Toronto Press, Toronto, Canada.
28. FAO, 1979. Manuals of food quality control. FAO Food and Nutrition paper 14/4.
29. Shewan, J.M., 1976. The bacteriology of fresh and spoiling fish and the biochemical changes induce by bacterial action. In: Proceeding of tropical institute conference on the handling, processing and marketing of tropical fish. Tropical Products Institute, London, UK, pp: 51-66.