Bangladesh J. Zool. **40**(1): 77-88, 2012

BACTERIOLOGICAL QUALITY OF MARKETED MOLA FISH, *AMBLYPHARYNGODON MOLA* FROM DHAKA METROPOLIS

Saima Sharif Nilla, Md. Anisur Rahman Khan[1], Md. Mahmudur Rahman Khan, Dewan Ali Ahsan and Md. Ghulam Mustafa*

Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh

Abstract: The bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen from three departmental chain shops of Dhaka metropolis were analyzed. The microbial quality parameters varied with different sources and the quality was found to be poor for local market fish samples. In total 24 samples were considered for bacteriological quality analysis and 10 pathogenic isolates for antibiotic sensitivity test to 12 antibiotics. The total bacterial count ranged from $1.8 \pm 0.25 \times 10^4$ to $6.5 \pm 0.75 \times 10^6$ cfu/g for fresh and $5.5 \pm 0.55 \times 10^3$ to $7.0 \pm 0.80 \times 10^5$ cfu/g for frozen mola. The highest total coliform count of mola was $8.0 \pm 0.55 \times 10^4$ and $6.1 \pm 0.40 \times 10^3$ cfu/g for local market and departmental chain shop, respectively. All fresh and frozen samples were observed having high quantity of *E. coli* above 10^2 cfu/g. Furthermore, *Salmonella-Shigella* was identified in 67% samples (75% of fresh and 58% of frozen samples) varied from $0.9 \pm 0.00 \times 10^2$ to $5.3 \pm 0.30 \times 10^3$ cfu/g whereas *Vibrio* spp. was confirmed in 79% samples (83% of fresh and 75% of frozen samples) of which 90% samples exceeded 10^2 cfu/g. Similar pattern was observed in *Staphylococcus* spp. with 83% of fresh and 58% of frozen samples (63% of total samples) beyond 10^3 cfu/g. In case of antibiotic sensitivity pattern of the indicator and pathogenic isolates, all of them were resistant to amoxicillin and penicillin. Most of the isolates were sensitive to bacitracin, ciprofloxacin, erythromycin and streptomycin. The findings proved that mola fish under this study was more or less contaminated and local fish market samples were highly privileged with food borne pathogens which confirmed the unhygienic condition of the market as well as the presence of antibiotic resistance bacteria in mola fish may threat to public health safety.

Key words: Bacteriological quality, *Amblypharyngodon mola,* Dhaka metropolis, Antibiotic sensitivity

INTRODUCTION

Bacteriological quality is of importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. Microbial hazards causing infections and poor health are closely related to food safety concerning with animal proteins derived from marketed food - fish, fishery products, meat and meat products. This creates a burning question for all consumers with a high risk commodity with regard to pathogenic bacterial contaminations alarming to food safety challenge. Food borne disease results from the ingestion of bacteria and the toxins produced by microorganisms present in the marketed

*Corresponding author: E-mail: mgmustafabd@yahoo.com; [1]Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.

food, and the intensity of the signs and symptoms may vary with the amount of the contaminated food ingested and susceptibility of the individuals to the toxin (Clarence *et al.* 2009).

Among Small Indigenous Species (SIS) of fish, mola (*Amblypharyngodon mola*) is most demanding and delicious fish to the people of Bangladesh. Mola is particularly important for its high content of vitamin-A than any other edible fish (Ahmed 1981). Nutrition surveys conducted in Bangladesh revealed that about 75% of the rural children in Bangladesh suffer from malnutrition and 25% of them below five years of age die due to malnutrition (Ahmed and Hassan 1983). Human nutritionists claim that small indigenous fish species especially mola can play a major role in the elimination of malnutrition as well as night blindness in rural Bangladesh.

The quality of the processed product largely depends on the quality of raw materials and it is difficult to preserve the freshness of raw materials when there is a long period of time between the harvesting and processing periods. During this period fishes continue to deteriorate due to improper handling and further processing can never bring back its freshness. Low quality frozen foods are related with improper processing and poor hygienic condition. Consequently, frozen fishes may be contaminated with different types of bacteria, such as *Vibrio cholerae*, *Salmonella*, coliform, fecal coliform, streptococci and *Staphylococcus aureus,* and these are responsible for causing cholera and other food borne diseases (Mobin *et al.* 2001). It is well documented that raw or frozen fishes provide important epidemiological pathways for food borne disease transmission (WHO 2002). So, the quality of the marketed fish is of major concerns to fish processors and public health authorities.

A live fish body is sterile and it is infected through contaminated water. During transportation of these fish to land and wholesale market, the fish may also infect people associated in handling. When the consumers purchase these fish, they (fish with high bacterial load) are definitely transferred to the person who carries out them (Das *et al.* 2007). Biological contaminants constitute the major cause of food borne diseases ranging from mild indisposition to chronic or life threatening illness (Phyllis 2007) and spoil food, including fish, meat, fresh fruits and vegetables (Pelczar *et al.* 1986).

Today more and more people are turning to fish as a healthy alternative to red meat. The low fat content of many fish species and the effects of the polyunsaturated fatty acids found in fatty fish species are extremely important aspects for health conscious people particularly in the affluent countries, where cardiovascular disease mortality is high (Huss 1994).

The objective of this study was to determine the bacteriological status of fresh mola fish collected from local markets and frozen mola fish from the departmental chain shops of Dhaka metropolis with a view to provide potential approaches for marketed food safety with regard to food borne pathogens.

MATERIAL AND METHODS

Sample collection: Mola samples were collected from different departmental chain shops as frozen and local retail markets as fresh (raw) at Dhaka metropolis in between April 2010 and December 2010. Total 24 samples, with 15-18 mola were taken in each sample, 12 samples from departmental chain shops and 12 from local markets were collected to assess the bacterial load. Identification was done according to Shafi and Quddus (2004) and Rahman (2005). The mola samples were collected by special sterile Ziploc bags to avoid further contamination and transported in an insulated box with ice to maintain the temperature (4°C to 6°C) and stored at –20°C at the laboratory until use (ICMSF 1998). The samples were processed and used within 24 hours of collection.

Processing of the samples: The samples were thawed at room temperature for 5-6 h to melt the ice. The whole fish were taken following the method of APHA (APHA 1998). The collected samples were separately homogenized with normal saline solution using a homogenizer. Pour plate or spread plate were prepared using 1 ml - 0.1 ml of the treated samples.

Microbiological analysis: The microbiological analysis was performed as per the standard methods adopted from Online Bacteriological Analytical Manual, USFDA for detection, enumeration and identification of individual organisms (BAM 2005).

Total bacterial count (TBC): Total bacterial count was obtained on nutrient agar (Becton Dickinson, France). The colonies were incubated for 24 h at 35-37^{0}C and calculated as cfu/g.

Total coliform (TC): Diluted samples were pour-plated on MacConkey agar (Oxoid Ltd., Hampshire, England); typical pink colonies for TC were counted after 24 h of incubation at 35-37^{0}C.

Fecal coliform (FC) and E. coli (EC): Dilutions made for TBC were pour-plated on MacConkey agar (Oxoid Ltd., Hampshire, England). Typical colonies were counted after 24 h of incubation at 35-37^{0}C. The suspected isolates were streaked on modified fecal coliform (mFC) agar plates and incubated at 44.5^{0}C for 24 h. Typical blue colonies were counted and was further confirmed by growing in eosin methylene blue (EMB) agar plates (Oxoid Ltd., Hampshire, England).

Salmonella-Shigella (SS): The sample (25 g) was homogenized in saline water. After the enrichment of the samples, a loopful of growth from the broth was streaked on Salmonella-Shigella agar (SSA; Oxoid Ltd., Hampshire, England), typical black colonies from plates were isolated and identified by biochemical tests.

Xylose Lysine Deoxycholate (XLD) (Oxoid Ltd., Hampshire, England) agar also used for the selective isolation of *Shigella* and *Salmonella* spp. The incubation period for *Shigella* and *Salmonella* spp. were 48 h at 35-37^{0}C.

Vibrio spp.: The sample (25 g) was homogenized in 225 mL of alkaline peptone water (APW) and incubated at 35^{0}C for 24 h. Diluted homogenates were pour-plated on TCBS agar (Oxoid Ltd., Hampshire, England).

Staphylococci: Diluted sample for TBC were spread-plated on mannitol salt agar (MSA) (Oxoid Ltd., Hampshire, England). Typical yellow colonies were counted after 48 h of incubation at 35^{0}C.

Biochemical tests: Biochemical tests were done according to the manual for general bacteriology of the American Society of Microbiology (ASM 1981). Oxidase test, catalase test, carbohydrate fermentation/utilization test, Kligler's iron agar (KIA) test, indole production test, methyl red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, nitrate reduction test, motility indole urea (MIU) tests, and salt tolerance (3%, 5%, 10% and 15% NaCl) test were done to identify the bacteria (Cappuccino and Sherman 1990).

Determination of antibiotic susceptibility of the isolated bacteria from mola samples: The susceptibility of *E. coli, Salmonella, Shigella, Vibrio* and *Staphylococcus* isolates to different antimicrobial agents was measured *in vitro* according to the Kirby-Bauer disk diffusion method (Bauer *et al.* 1966). Commercially available 12 antimicrobial discs [ampicillin (AMP), amoxicillin (AML), bacitracin (B), chloramphenicol (C), ciprofloxacin (CIP), erythromycin (E), gentamycin (GN), kanamycin (K), penicillin (P), polymyxinB (PB), streptomycin (S) and tetracycline (TE)] were used for the test.

Statistical analysis: Statistical analysis was performed with the SPSS software package (verson11.5, SAS Institute Inc, Cary, USA).

RESULTS AND DISCUSSION

The results of bacteriological quality analysis conducted on collected 24 fresh (sample 1-12) and frozen (sample 13-24) mola fish samples, respectively from three different local fish markets (LFM) and three different departmental chain shops (DCS) are shown in Table 1. The results reveal that bacterial count was higher in the local markets samples.

The TBC of mola fish collected from Dhaka metropolis varied from 10^3 to 10^6 cfu/g, with 62.5% samples were found cross the limit (> 10^5 cfu/g) set by International Commission on Microbiological Specifications for Foods (ICMSF 1986). The highest bacterial abundance ($6.5 \pm 0.75 \times 10^6$) was found in sample 8 from LFM-2 and the lowest load ($5.5 \pm 0.55 \times 10^3$) was in sample 22 from DCS-3. The bacterial density in fish apparently gives an idea about the quality of the samples. The processed food or food products are considered as spoiled when the TBC values reach to 10^6 cfu/g or more in food items (Shewan 1970). So, this result proves that the fish quality of departmental chain shops was better than those of local markets in respect of bacterial load.

Table 1. Total bacterial count (TBC), total coliform (TC), fecal coliform (FC) and *E. coli* (EC) density (mean ± SEM) in mola fish samples from different local fish markets (LFM) and different departmental chain shops (DCS) of Dhaka metropolis.

Sample No		Bacterial density (cfu/g)			
		TBC	TC	FC	EC
Local fish markets					
LFM-1	1	$1.8 \pm 0.25 \times 10^4$	$4.0 \pm 0.20 \times 10^3$	$1.7 \pm 0.15 \times 10^3$	$1.4 \pm 0.15 \times 10^3$
	2	$2.0 \pm 0.30 \times 10^4$	$1.1 \pm 0.25 \times 10^4$	$2.7 \pm 0.30 \times 10^3$	$1.7 \pm 0.15 \times 10^3$
	3	$1.2 \pm 0.35 \times 10^5$	$5.6 \pm 0.30 \times 10^3$	$2.7 \pm 0.35 \times 10^4$	$3.1 \pm 0.25 \times 10^3$
	4	$5.5 \pm 0.35 \times 10^4$	$8.5 \pm 0.45 \times 10^3$	$4.8 \pm 0.45 \times 10^4$	$4.7 \pm 0.45 \times 10^4$
LFM-2	5	$7.5 \pm 0.55 \times 10^5$	$2.4 \pm 0.30 \times 10^3$	$3.5 \pm 0.10 \times 10^2$	$3.4 \pm 0.10 \times 10^2$
	6	$1.2 \pm 0.35 \times 10^5$	$9.0 \pm 0.50 \times 10^3$	$6.0 \pm 0.40 \times 10^3$	$4.8 \pm 0.45 \times 10^4$
	7	$5.2 \pm 0.45 \times 10^5$	$5.2 \pm 0.30 \times 10^3$	$2.5 \pm 0.35 \times 10^4$	$2.9 \pm 0.35 \times 10^4$
	8	$6.5 \pm 0.75 \times 10^6$	$1.2 \pm 0.30 \times 10^4$	$4.0 \pm 0.40 \times 10^4$	$3.9 \pm 0.40 \times 10^4$
LFM-3	9	$7.1 \pm 0.55 \times 10^5$	$8.1 \pm 0.45 \times 10^3$	$4.1 \pm 0.40 \times 10^4$	$3.8 \pm 0.40 \times 10^4$
	10	$5.1 \pm 0.45 \times 10^5$	$7.0 \pm 0.50 \times 10^4$	$7.6 \pm 0.15 \times 10^2$	$4.1 \pm 0.30 \times 10^3$
	11	$5.6 \pm 0.50 \times 10^5$	$8.0 \pm 0.55 \times 10^4$	$1.9 \pm 0.20 \times 10^3$	$1.9 \pm 0.20 \times 10^3$
	12	$4.3 \pm 0.60 \times 10^6$	$4.4 \pm 0.45 \times 10^4$	$3.6 \pm 0.25 \times 10^3$	$5.1 \pm 0.30 \times 10^3$
Departmental chain shops					
DCS-1	13	$5.8 \pm 0.70 \times 10^5$	$2.3 \pm 0.25 \times 10^3$	$1.6 \pm 0.15 \times 10^3$	$1.8 \pm 0.10 \times 10^2$
	14	$6.0 \pm 0.60 \times 10^4$	$2.0 \pm 0.10 \times 10^2$	$2.0 \pm 0.25 \times 10^3$	$1.8 \pm 0.20 \times 10^3$
	15	$6.1 \pm 0.75 \times 10^5$	$1.7 \pm 0.15 \times 10^3$	$2.4 \pm 0.25 \times 10^3$	$2.5 \pm 0.25 \times 10^3$
	16	$6.5 \pm 0.75 \times 10^5$	$3.2 \pm 0.25 \times 10^3$	$1.2 \pm 0.10 \times 10^2$	$2.1 \pm 0.25 \times 10^3$
DCS-2	17	$6.1 \pm 0.75 \times 10^5$	$3.5 \pm 0.25 \times 10^3$	$2.7 \pm 0.30 \times 10^3$	$1.7 \pm 0.10 \times 10^2$
	18	$5.9 \pm 0.70 \times 10^5$	$3.1 \pm 0.25 \times 10^3$	$2.1 \pm 0.10 \times 10^2$	$2.4 \pm 0.25 \times 10^3$
	19	$6.9 \pm 0.80 \times 10^4$	$6.1 \pm 0.40 \times 10^3$	$2.1 \pm 0.25 \times 10^3$	$1.4 \pm 0.10 \times 10^2$
	20	$7.0 \pm 0.80 \times 10^4$	$2.0 \pm 0.20 \times 10^3$	$1.5 \pm 0.10 \times 10^2$	$2.3 \pm 0.25 \times 10^3$
DCS-3	21	$5.3 \pm 0.65 \times 10^5$	$4.2 \pm 0.30 \times 10^3$	$1.0 \pm 0.10 \times 10^2$	$1.9 \pm 0.10 \times 10^2$
	22	$5.5 \pm 0.55 \times 10^3$	$2.7 \pm 0.15 \times 10^2$	$1.9 \pm 0.10 \times 10^2$	$1.5 \pm 0.10 \times 10^2$
	23	$7.4 \pm 0.65 \times 10^4$	$1.6 \pm 0.15 \times 10^3$	$1.5 \pm 0.10 \times 10^2$	$1.7 \pm 0.20 \times 10^3$
	24	$7.0 \pm 0.65 \times 10^4$	$1.4 \pm 0.15 \times 10^3$	$1.3 \pm 0.15 \times 10^3$	$2.8 \pm 0.25 \times 10^3$

The contaminated source of water, poor hygiene and sanitation condition of the processing or market premises might be the causes for such contagion

(Hatha *et al.* 2003) and high microbial abundance (Nilla *et al.* 2012). The bacterial flora on newly caught fish depends on the environment in which it is caught rather than on the fish species (Shewan 1961). Another source of contamination of harmful microorganisms could be catching vessels (Wahab *et al.* 2003). Besides, freezing only can limit the growth of microbes for a little period and when favorable condition comes back, most of the microbes can multiply within short time (Leita˜O and Rios 2000).

All samples were observed having high quantity of total coliform exceeding the limit (> 10^2 cfu/g) suggested by ICMSF (ICMSF 1986) and proves the supply of low quality fish in either local fish markets or departmental chain shops. Table 1 shows the highest and lowest TC density were found 8.0 ± 0.55 × 10^4 and 2.0 ± 0.10 × 10^2 cfu/g in sample 11 from LFM-3 and sample 14 from DCS-1, respectively. The presence of TC confirms the sewage contagion. It also indicates the contamination during handling and selling processes in the markets including holding temperature. Moreover, the contamination may also come from the water use for washing or icing (Boyd 1990).

Fecal coliform were more accurate indication of animal or human waste than the total coliform because the origins of FC are more specific than the origins of TC group of bacteria (CDCP 2010). Among the 24 samples, the highest FC count was observed 4.8 ± 0.45 × 10^4 cfu/g in sample 4 from LFM-1, whereas the lowest was 1.0 ± 0.10 × 10^2 cfu/g in sample 21 from DCS-3 (Table 1). This result reveals lower contamination in the departmental chain shops than the local fish markets. The FC contents in mola also refer to the poor sanitary and hygienic conditions of the markets and fish landing centers.

E. coli is usually considered as an indicator of fecal contamination. The highest load of EC was 4.8 ± 0.45 × 10^4 cfu/g and the lowest was 1.4 ± 0.10 × 10^2 cfu/g found in sample 6 from LFM-2 and sample 19 from DCS-2, respectively (Table 1). The presence of EC in higher range suggests the contamination of the samples before or during handling, processing and marketing. This result indicates that the water or processing units were somehow contaminated with human or animal waste. Although the water cannot be linked directly to the contamination by human sewage, since the bacteria is found in high concentration within the sewage.

Since, all of the fish samples were found exceeded the limit of 1.0 x 10^2 cfu/g according to ICMSF (ICMSF 1986), further identification was carried out to investigate the presence of other harmful and pathogenic microorganisms, such as *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Staphylococcus* spp.

The *Salmonella-Shigella* was identified in 67% (16 out of total 24) of the total samples (Table 2) where 75% of the fresh samples and 58% of the frozen

samples were infected by SS. The SS count ranged between 0.9 ± 0.00 ×10^2 and 5.3 ± 0.30 × 10^3 cfu/g for the fish samples that also exceeded the limit of 1.0 × 10^2 cfu/g. The highest and lowest levels of SS were found in sample 7 from LFM-2 and sample 20 from DCS-2, respectively. This result proves that the processing, handling, storage condition, hygiene and sanitary maintenance of departmental chain shops was good and the quality of fish was better than the local markets. Besides, the relatively long storage period with low ice quality until sold and improper storage condition due to handling disruption might be the reasons for the SS infection in local markets fish (Nilla *et al.* 2012). Moreover, as the marketed mola fish either fresh or frozen directly came from different sources of Dhaka metropolis, so the fish might contain SS isolates (Reilly *et al.* 1992).

Table 2. *Salmonella-Shigella (SS), Vibrio* spp. and *Staphylococcus* spp. abundance (mean ± SEM) in mola fish samples from different local fish markets (LFM) and different departmental chain shops (DCS) of Dhaka metropolis.

Source		Sample No	Bacterial density (cfu/g)		
			SS	*Vibrio* spp.	*Staph.* spp.
Local fish markets	LFM-1	1	1.8 ± 0.15 × 10^3	2.0 ± 0.20 × 10^3	2.7 ± 0.25 × 10^3
		2	2.8 ± 0.25 × 10^3	3.5 ± 0.25 × 10^3	2.5 ± 0.25 × 10^3
		3	1.8 ± 0.10 × 10^2	NF[a]	1.6 ± 0.15 × 10^3
		4	NF	1.5 ± 0.20 × 10^3	1.7 ± 0.15 × 10^3
	LFM-2	5	2.5 ± 0.25 × 10^3	2.2 ± 0.15 × 10^2	2.6 ± 0.15 × 10^2
		6	2.9 ± 0.25 × 10^3	2.7 ± 0.25 × 10^3	1.9 ± 0.20 × 10^3
		7	5.3 ± 0.30 × 10^3	3.1 ± 0.20 × 10^2	1.3 ± 0.15 × 10^3
		8	NF	2.5 ± 0.20 × 10^3	1.8 ± 0.15 × 10^3
	LFM-3	9	1.0 ± 0.10 × 10^2	1.7 ± 0.15 × 10^3	1.7 ± 0.15 × 10^3
		10	3.1 ± 0.25 × 10^3	NF	4.1 ± 0.40 × 10^4
		11	NF	1.3 ± 0.10 × 10^2	4.6 ± 0.10 × 10^2
		12	1.2 ± 0.10 × 10^2	1.9 ± 0.15 × 10^2	2.1 ± 0.20 × 10^3
Departmental chain shops	DCS-1	13	1.5 ± 0.10 ×10^2	1.4 ± 0.10 × 10^2	3.4 ± 0.20 × 10^2
		14	NF	NF	2.0 ± 0.15 × 10^2
		15	2.4 ± 0.15 × 10^2	1.2 ± 0.10 × 10^2	3.7 ± 0.20 × 10^2
		16	1.2 ± 0.10 × 10^2	2.2 ± 0.15 × 10^2	3.3 ± 0.35 × 10^3
	DCS-2	17	NF	2.0 ± 0.15 × 10^2	3.7 ± 0.40 × 10^3
		18	1.4 ± 0.10 × 10^2	2.3 ± 0.15 × 10^2	3.9 ± 0.40 × 10^3
		19	NF	NF	5.0 ± 0.20 × 10^2
		20	0.9 ± 0.00 × 10^2	NF	2.5 ± 0.25 × 10^3
	DCS-3	21	NF	1.3 ± 0.10 × 10^2	3.0 ± 0.30 × 10^3
		22	1.0 ± 0.10 ×10^2	1.2 ± 0.10 × 10^2	1.7 ± 0.15 × 10^3
		23	NF	0.6 ± 0.00 × 10^2	1.6 ± 0.20 × 10^2
		24	1.1 ± 0.10 × 10^2	1.0 ± 0.10 × 10^2	3.2 ± 0.30 × 10^3

[a]NF = Not Found

The environment acts as main source of SS in aquaculture products rather than poor standards of hygiene and sanitation. But external contamination may also be the source of the occurrence of these bacteria in fish (Huss 1994). Besides, FC is present highly in diarrheal stools of infected persons. So, the unwashed hands of infected food handlers forgetting to wash hands with soap after using the bathroom may also contaminate food (CDCP 2010). Fish cooking prior to consumption causes negligible health risks to the consumers for cross contamination in the kitchens.

The presence of *Vibrio* spp. in mola fish is of great public health concern as they may cause of infection to the consumers. In the present study, 19 out of 24 (79% of total) samples where 83% of fresh and 75% of frozen samples were found to have *Vibrio* spp. The highest load was found in sample 2 ($3.5 \pm 0.25 \times 10^3$ cfu/g) from LFM-1and the lowest density was $0.6 \pm 0.00 \times 10^2$ cfu/g in sample 23 from DCS-3 (Table 2). As 89.5% of *Vibrio* spp. containing samples were found above the limit ($> 10^2$ cfu/g) according to ICMSF (ICMSF 1986), this study revealed that microbial quality of local markets was not good due to the presence of *Vibrio* spp. in most samples.

It was very remarkable to find out *Vibrio* spp. in frozen samples because *Vibrio* normally cannot survive in the frozen condition due to the absence of moisture (Jay 1996). Here, the cross contamination with other frozen foods, i.e. shrimp, meat etc., power supply disruption, inadequate freezing condition and the presence of moisture during freezing might be probable reasons for survival of *Vibrio* spp. in frozen fish samples. As the frozen fish already contained the *Vibrio* spp., they revived themselves easily during the study and developed (Rahman *et al*. 2009). Besides, *Vibrio* spp. is mainly present in the intestine of the fish, so the density was found high because the whole fish was considered for microbial analysis (Nilla *et al*. 2012).

There were considerable numbers of *Staphylococcus* spp. found in almost all experimented samples (Table 2). The highest load of *Stephylococcus* was $4.1 \pm 0.40 \times 10^4$ cfu/g while the lowest was $1.6 \pm 0.20 \times 10^2$ cfu/g in sample 10 from LFM-3 and 23 from DCS-3, respectively. In this study, 83% of fresh and 58% of frozen samples (63% of total samples) were observed crossing the suggested limit ($> 10^3$ cfu/g) by ICMSF (ICMSF 1986). This result confirms the contagion of the frozen samples with *Staphylococcus* via infected food handlers or from the environment. The infected individual with an infection on hands or with a cold or sore throat more often acts as the contamination source in food. In contrast, rapid growth and toxin production can take place in mola fish if re-contamination with *Staphylococcus* is taken place (Hatha *et al.* 2003).

The present study revealed the incidence of antibiotic resistance in the bacterial strains isolated from mola fish. Table 3 summarizes the antibiotic sensitivity pattern of four *E. coli,* four *Salmonella-Shigella* and two *Staphylococcus* isolates to 12 antimicrobial agents tested in this study. The results show that all of the 10 isolates were resistant against AML and P; frequently sensitive to B, CIP, E and S in the sensitivity test. Among the four isolates of EC, three were resistant to PB and TE but all were sensitive to E. Three SS isolates were also sensitive to B, S and two were resistant to TE, whereas both *Staphylococcus* isolates were resistant to PB and two were sensitive to K (Table 3). This study clearly demonstrated the potential risk of the abusive use of antibiotics as bactericidal, fungicidal etc. (Rahman *et al.* 2009).

Table 3. Antibacterial sensitivity of *E. coli* (EC), *Salmonella-Shigella* (SS) and *Staphylococcus* isolates from the fresh and frozen fish samples.

Isolated strains	Resistant	Intermediate	Sensitive
EC-1	P, PB, AML, TE	AMP	GN, E, K, S, CIP
EC-2	PB, P, AML	E, GN, TE	B, K, E, CIP
EC-3	K, AML, C, P, TE	Nil	E, B, GN, S, AMP
EC-4	P, AML, PB, TE	C, GN	CIP, S, B, E
SS-1	P, AML, GN	AMP, TE	K, S, B, CIP
SS-2	P, GN, AML	AMP, S	PB, TE, K, E, B
SS-3	P, AML, TE	AMP, PB, GN	S, B, CIP, E
SS-4	AML, P, K, TE, B	Nil	S, E, C, CIP, PB
Staph-1	P, C, CIP, PB, AML	Nil	GN, K, B, E, AMP
Staph-2	AML, AMP, P, PB	C, E	S, CIP, K, TE

AMP = ampicillin, AML = amoxicillin, B = bacitracin, C = chloramphenicol, CIP = ciprofloxacin, E = erythromycin, GN = gentamycin, K = kanamycin, P = penicillin, PB = polymyxinB, S = streptomycin and TE = tetracycline

Multiple antibiotic resistance (MAR) have been reported in fish pathogen and bacteria from aquaculture environment with a variety of drug or an uncertain antibiotic usage history (Ghosh and Mandal 2010). The high level of water contamination with the industrial effluents and agricultural pollutants may magnify the exchange possibilities. Besides, the widespread use of antibiotics in the aquaculture systems and agricultural sectors in Bangladesh may act as the source of antibiotics diffusion into the sediment. The uncontrolled antibiotics will remain in the sediment and an alternation of micro flora composition of the sediment and antibiotic-resistant bacteria (ARB) may occur with exerting of selective pressure (Sorum 2006). So this result indicates that the uncontrolled and irregular use of antibacterial agents in aquaculture systems and agricultural sectors is responsible for the occurrence of the MAR traits among the fish pathogens and the majority of the ARB carry drug resistant (R) factor

(Keys *et al.* 1986). This result also suggests that commercial fish may act as the reservoir for MAR and facilitate the dissemination of the ARB (Ryu *et al.* 2012).

Concluding remarks: This study provides a clear perspective of the bacterial profusion in marketed mola fish. It also revealed that the fish samples from departmental chain shops were comparatively less contaminated with bacteria than those of local fish markets due to their airtight display tray with sufficient ice and careful handling. The higher total bacteria and coliform count in local market samples indicated the unhygienic condition of the processing and marketing area. The main reasons for infecting the local marketed mola might be rough handling and sorting or lack of sanitation. For frozen fish, the fluctuation of storage temperature due to power interruption might be the major cause. Cross contamination with other frozen foods, i.e. shrimp, meat etc. might also be another reason for bacterial contagion in frozen mola fish. Overall, the sub-tropical environment might also be the crucial reason for bacterial contamination in both fresh and frozen mola fish. Moreover, the antibiotic resistance of bacteria is a significant public health problem. The result suggests that bacterial abundance and presence of antibiotic resistance bacteria into mola fish sold in the local markets of Dhaka metropolis may create ecological and public health implications as well as are of special concern. The retailers and handlers should take proper training on the aspect to avoid the health risks and cross contamination. Consumers also could wash the fish with water cautiously prior to cook. This study also emphasizes the necessity of increasing awareness about the use of antibiotics in aquaculture sectors to ensure the absence of drug-resistant pathogenic microorganisms in fish for achieving the food microbiological safety.

Acknowledgements: This study was supported by National Science, Information and Communication Technology (NSICT) Fellowship from Ministry of Science, Information and Communication Technology, Government of Bangladesh. The authors also acknowledged thankfully about the facilities provided by the Department of Fisheries and the Department of Microbiology, University of Dhaka.

LITERATURE CITED

AHMED, K. 1981. Nutritional blindness in Bangladesh. In Touch: *VHSS News letter* **45**: 1-2.

AHMED, K. and HASSAN, N. 1983. *Nutrition Survey of Rural Bangladesh 1981-1982*. Institute of Nutrition and Food Science, University of Dhaka, Bangladesh. 15 pp.

APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*. (ed. Clesceri, L.S., Greenboug, L.S. and Eaton, A.D.). Washington, DC: American Public Health Association/ American Water Works Association/ Water Environment Federation.

ASM. 1981. Manual for General Bacteriology. American Society of Microbiology. Website: http://ourfood.com/General bacteriology.html. Accessed on 15-04-2010.

BAM. 2005. USFDA for Detection, Enumeration and Identification of Individual Organisms. Bacteriological Analytical Manual Website: http://www.foodinfonet.com/publication/fdaBAM.html. Accessed on 17-04-2010.

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C. and TURCK, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.* **45**: 493-496.

BOYD, C.E. 1990. *Water Quality in Ponds for Aquaculture*. Birmingham Publishing Co., Alabama, USA. 480 pp.

CAPPUCCINO, J.G. and SHERMAN, N. 1990. *Microbiology: A Laboratory Manual*. 4th ed. 137-183 pp.

CDCP. 2010. *Escherichia coli*. Center For Disease Control and Prevention, FAQ. Website: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.html. Accessed on 10-05-2010.

CLARENCE, S.Y., OBINNA, C.N. and SHALOM, N.C. 2009. Assessment of bacteriological quality of ready to eat food in Benin city metropolis, Nigeria. *Afr. J. Microb. Res.* **3**(6): 390-395.

DAS, M., AHMED, K.M. and PARVEEN, S., 2007. Microbiological analysis of some raw fish samples. *Bangladesh. J. Microbiol.* **24**(1): 67-69.

GHOSH, K. and MANDAL, S. 2010. Antibiotic resistant bacteria in consumable fishes from Digha coast, West Bengal, India. *Proc. zool. Soc.* **63**(1): 13-20.

HATHA, M.A.A., MAQBOOL, T.K. and KUMAR, S. 2003. Microbial quality of shrimp products of export trade produced from aquacultured shrimp. *Int. J. Food Microbiol.* **5**(2): 82, 213-221.

HUSS, H.H. 1994. *Assurance of Seafood Quality*. FAO Fisheries Technical Paper 334. Food and Agriculture Organization, Rome. 94, 97, 169 pp.

ICMSF (INTERNATIONAL COMMISSION ON MICROBIAL SPECIFICATION FOR FOODS). 1998. *Sampling for Microbiological Analysis: Principles and Specific Applications*. University of Toronto Press, Toronto, Canada. Vol. *2*. 142 pp.

ICMSF. 1986. Microorganisms in Foods. In: *Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality*. 2nd ed. Blackwell Scientific Publications. 42, 181-195 pp.

JAY, J.M. 1996. *Modern Food Microbiology*. 4th ed. Maryland: Aspen Publishers Inc. 583-585 pp.

KEYS, A., ADERSON, J.T. and GRANDE, F. 1986. Serum cholesterol response to changes in the diet. *Metabolism* **14**(7): 776-787.

LEITA˜O, M.F.F. and RIOS, D.P.A. 2000. Microbiological and chemical changes in freshwater prawn stored under refrigeration. *Braz. J. Microbiol.* **13**(3): 31, 3-7.

MOBIN, S.M.A., CHOWDHURY, M.B.R., ISLAM, M.S. and UDDIN, M.N. 2001. Status of bacterial flora in the intestine of two freshwater fish. *Bangladesh J. Life Sci.* **13**(1&2): 149-155.

NILLA, S.S., MUSTAFA, M.G., AHSAN, D.A., KHAN, M.M.R. and KHAN, M.A.R. 2012. Bacterial abundance in Indian white shrimp, *Penaeus indicus* collected from two different market conditions of Dhaka city. *Dhaka Univ. J. Biol. Sci.* **21**(1): 29-38.

PHYLLIS, E. 2007. *Food Safety: New Perspectives*. ASM Press. Virginia, USA. 414 pp.

PELCZAR, M.J., CHAN, E.C.S. and KRIEG, N.R. 1986. *Microbiology*. 5th ed. McGraw Hill, Inc. pp. 261-333, 488-510.

RAHMAN, A.K.A. 2005. *Freshwater Fishes of Bangladesh*. 2nd ed. Zool. Soc. Bangladesh, Dhaka, Bangladesh, pp. 122-123.

RAHMAN, M., RAHMAN, M.Z., AKHTER, H., BEGUM, A. and KHAN, S.I. 2009. Occurrence and characterization of *Vibrio* spp. in raw and frozen shrimps. *Bangladesh J. Zool.* **37**(2): 247-255.

REILLY, P.J.A., TWIDDY, D.R. and FUCHS, R.S. 1992. *Review of the Occurrence of Salmonella in Cultured Tropical Shrimp*. FAO Fish. Circ. No. 851. FAO, Rome, Italy.

RYU, S.H., PARK, S.G., CHOI, S.M., HWANG, Y.O., HAM, H.J., KIM, S.U., LEE, Y.K., KIM, M.S., PARK, G.Y., KIM, K.S. and CHAE, Y.Z. 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* strains from commercial fish and seafood. *Int. J. Food Microbiol.* **15**(2): 14-18.

SHAFI, M. and QUDDUS, M.M.A. 2004. *Bangladesher Matsho Shampod (Fisheries of Bangladesh).* 2nd ed. Kabir Publications, Dhaka. 144-145 pp.

SHEWAN, J.M. 1970. *Bacteriological Standards for Fish and Fishery Products, Chemistry and Industry*. Academic press, New York. 193 pp.

SHEWAN, J.M. 1961. The Microbiology of Seawater Fish. In: *Fish as Food Vol. I* (ed. Brogstrom, G.). Academic press, New York. 487 pp.

SORUM, H. 2006. Antimicrobial Resistance in Bacteria of Animal Origin. In: *Antimicrobial Drug Resistance in Fish Pathogens*. Chapter 13 (ed. Aarestrup, F.M.). American Society for Microbiology Press: Washington, DC, USA. pp. 213-238.

WAHAB, M.A., BERGHEIM, A. and BRAATEN, B. 2003. Water quality and partial mass budget in extensive shrimp ponds in Bangladesh. *Science Direct (Internet ed.).* pp. 218-423.

WHO. 2002. Food borne Disease, Emerging. World Health Organization. Fact Sheet No. 124. Website: http://www.who.int/mediacentre/factsheets/fs124/en/. Accessed on 25-08-2010.

(Manuscript received on December 20, 2011; revised on April 1, 2012)