

## Bacteriological study of cultured silver carp (*Hypophthalmichthys molitrix*) in Gilan province, Iran

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### Abstract

The study was undertaken to determine bacterial contamination of warm-water fish ponds and silver carps harvested from those ponds in Guilan province, Iran. In this respect, water samples were taken from 7 ponds and investigated by testing total bacteria, coliforms, and *Escherichia coli*. In addition, skin swab samples of silver carps caught from each pond were studied for enumeration of the same bacteria as well as *Staphylococcus aureus* and also for the presence of some human bacterial pathogens including *Salmonella* spp., *Vibrio* spp., and *Listeria monocytogenes*. For all bacteriological examination techniques of International Organization for Standardization were followed. Results showed that mean water bacterial quality in ponds was significantly different ( $p < 0.01$ ). About 43% of water samples were positive for *E. coli*, which indicates fecal contamination of some fish ponds. Bacteriological results of the fish harvested from ponds showed significant differences ( $p < 0.0001$ ) in total bacterial counts and coliform counts. However, counts of *E. coli* and *S. aureus* did not vary significantly ( $p > 0.05$ ) in different ponds. Results also showed that potential human pathogens were isolated from about 78.5% of fish studied. Out of five bacteria genera investigated, *S. aureus*, *E. coli*, *V. cholera*, and *L. monocytogenes* were isolated from 78.57%, 47.61%, 7.14%, and 4.76% of the fish samples, respectively. The *Salmonella* spp. and *V. parahaemolyticus* were not detected in fish samples during the study. The association of pathogenic bacteria among silver carps suggests that if fish are handled or prepared improperly it could lead to food safety hazards for consumers.

**Keywords:** Warm-water fish pond, Silver carp, Food safety hazards

## Introduction

Fish are considered as safe, nutritious and beneficial food. However, aquaculture yields can be associated with certain food safety issues (WHO, 2007). The importance of aquaculture continues to expand, especially for freshwater species. Based on FAO (2012), almost one third of fish used for human consumption are produced in aquaculture. Increasing demand for fish and seafood has had an increase trend in recent years with annual growth rate of 8.5%. This trend is likely to be continued, with the contribution of aquaculture to fish food supply estimated to reach 60% by 2020 (Washington and Ababouch, 2011).

One of the essential things in food hygiene is the examination of food, especially for the presence of microorganisms. This is very much needed for the protection and maintenance of community health (Sujatha et al., 2011). Fish are often infected with or may harbor various bacteria which may cause health hazards to the fisherman, fish handlers and even to the consumers (Monzur-Hassan et al., 1994). Several studies have demonstrated many bacteria species encountered in different fish which are potentially pathogenic (Feldhusen, 2000; Razavilar, 2002; Phong Lan et al., 2007; Ekpo et al., 2010; Sujatha et al., 2011).

The level of bacterial contamination in fish at the time of capture and the nature of the pathogenic bacteria in fish depends on many factors such as the bacteriological quality of the harvest water, natural conditions when fish is captured, handling practices and some

other factors which influence the quality and safety of the fish (Jayasinghe and Rajakaruna, 2005; Reynisson et al., 2009; CAC, 2010; FDA, 2011). Microbial quality of farmed fish is largely determined by the quality of the water in which they were cultivated (Buras, 1993; Ekpo et al., 2010). The edible muscle tissue of finfish is normally sterile at the time of capture and bacteria are usually present on the skin, gills and in the intestinal tract (CAC, 2010). However, some bacteria such as *Staphylococcus* spp. may be found only in the body surface (Ekpo et al., 2010). The microbial count in different fishes have showed that the number of organisms were more on the body surface of big fishes with higher body weight than on the surface of small fishes, because the organisms use the surface area of the fish as a microhabitat in their ecosystem (*ibid.*). After fish is being caught and dying, the immune system collapses and bacteria are allowed to proliferate freely on the skin surface (Emikpe et al., 2011). However, the type of microorganism found associated with a particular fish depends on the water it was found. Freshly harvested aquaculture products, particularly those from tropical regions, may harbor pathogenic bacteria which form part of natural micro-flora of fish ponds (Claucas and Ward, 1996). Thus, water and fish quality are very important subjects to protect public health with a special emphasis on the microbiology.

Human pathogens have been reported to be associated with different types of fish (Abou-Elela and Farag,

2004). The fish that live in water polluted with human and the animal fecal matter may carry considerable numbers of bacteria such as *Salmonella*, *V. cholera*, *E. coli* and other Coliforms (Jayasinghe and Rajakaruna, 2005). Human infections and intoxications caused by pathogens transmitted from fish are quite common and the following bacteria have been recorded: *Salmonella* spp., *Escherichia coli*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Staphylococcus aureus* and *Listeria monocytogenes* (Novotny et al., 2004). Outbreaks usually occur due to the consumption of raw or insufficiently heat-treated fish, which may be contaminated with bacteria from water environment (*Vibrio* spp.) or terrestrial sources (*Salmonella* spp., *Staphylococcus* spp., *V. cholerae*) (Novotny et al., 2004). In the case of poor hygiene, the contamination of fish and fish products may increase due to unsanitary procedures, the rotation of the assigned duties of workers (*ibid.*).

Carp species such as silver carp are one of the most important economical and widely cultivated warm water fish in Iran which is widely sold and used in its fresh (Salehi, 2006, 2009). The aim of this study was to investigate some bacterial contamination of water in warm-water fish ponds and silver carps cultivated in those ponds in Guilan province, Iran.

### Materials and methods

The study was carried out in seven warm-water fish ponds (named from A to G) which were randomly selected from different localities of Guilan province,

north of Iran through the harvesting season in winter. The ponds were located in four main regions. The water resource of ponds was mainly supplied by Sefidrood River, except for A and B ponds which were mainly supplied by underground waters. Four species of warm-water fish including common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), and grass carp (*Ctenopharyngodon idella*) were cultured in those ponds from which only silver carp was considered in this study.

In the first step, water in fish ponds was aimed to investigate. Water samples were then collected from three sites of each pond (inlet, middle, and outlet) from about 10 to 30 cm below the surface with separate sterile plastic containers and then were pooled as a representative sample. Another representative sample was taken in the same way, so that we had two samples from each pond for bacterial examinations. The water temperature of fish ponds ranged from 8 – 12°C and pH from 7.3 – 8.1. After collection, the water samples were sent to the laboratory within 12 h. They were kept at 4 – 5°C during the transfer and examined within 24 h of sampling. In the laboratory, each sample was first blended with vortex apparatus for 10 seconds. Then for enumerating total bacteria, coliforms, and *E. coli*, serial decimal dilution of the water sample was prepared by adding 1 ml of sample in sterile test tube, containing 9 ml of sterile peptone water solution and this 1-in-10 dilution was then homogenized with vortex apparatus for 10 seconds. This

dilution was then used for bacteriological analyses directly or diluted further in the same way. The bacteriological techniques of the International Organization for Standardization (ISO) including ISO 4833 (2003), ISO 4832 (2006), and ISO 7251 (2005) were used for enumeration of total bacteria at 25 °C, coliforms at 30 °C, and *E. coli* at 44 °C, respectively. Results expressed in counts of cell-forming units (CFU) per ml of fish ponds' water.

In the next step, silver carps which were captured from the studied ponds were sampled through the harvesting season, before being sent to the market. In this respect, at the same time which the water samples were collected, six silver carps were randomly collected immediately after they had been harvested from each warm-water fish pond. Those chosen for this study had average length from 45 to 50 cm and average weight from 2 to 2.5 kg. Samples from skin of silver carps were taken by sterile cotton tipped swab from each fish from external surface using the methods described by American Public Health Association (APHA) (Nickelson et al., 2001). Skin samples were taken from a 50 cm<sup>2</sup> central area of the fish by marking out, using a sterile template. Swab sampling were conducted as soon as possible after the fish were caught, then the swabs were placed and shaken each in tubes containing 5 ml of Phosphate buffer saline (Pbs), separately and sent to laboratories for bacteriological examinations. The swab samples were transported to the laboratory within 12 h and were kept at 4 – 5°C during the transfer. There, each sample was first

blended with vortex apparatus for 10 seconds and serial decimal dilution was made of each fish swab sample using the peptone water diluents. Then bacteriological examinations was performed to enumerate total bacteria, coliforms, *E.coli*, and *Staphylococcus aureus* and to detect the presence of *Salmonella* spp., *Vibrio cholera*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes*. In this respect, swab samples were cultured on general and selective media for enumeration and detection of studied bacteria by using the ISO bacteriological techniques (ISO 4833: 2003, ISO 4832: 2006, ISO 7251: 2005, ISO 6888-1: 1999, ISO 6579: 2002, ISO/TS 21872-1: 2007, and ISO 11290-2: 1998), within 24 h of sampling. The isolates were identified by series of biochemical and physiological tests according to the related ISO procedure for that bacterium. For examination of total bacteria and coliforms, the related plates were aerobically incubated at 25 °C and 30 °C, respectively. Results expressed in counts of CFU per cm<sup>2</sup> of fish skin or detection of bacteria per surface area of sampling.

It is worth mentioning that all the used materials during water sampling of fish ponds and swab sampling of silver carps were sterile or had been sterilized before sampling processes. Media used for detection and confirmatory tests, were purchased from Merck, and prepared according to the manufacturer's instructions.

All of the results are presented as mean values. One-way ANOVA was then

applied to statistically analyze data, and if there was a significant difference ( $p < 0.05$ ), the calculated mean values were compared using Duncan's Multiple Range test. The Pearson Correlation Coefficients was used to compare the bacterial counts results from the fish swab samples. The data were analyzed with SAS software.

### Results

The results in Table 1 show means of bacterial counts of studied warm-water ponds. Significant differences ( $p < 0.01$ ) were observed among the bacterial quality of the water in the seven ponds. The bacterial quality of ponds is shown in the same table based on Duncan's test. The water quality in pond E had higher bacterial counts of total bacteria ( $3.42 \times 10^4$  cfu ml<sup>-1</sup>), coliform bacteria ( $1.03 \times 10^3$  cfu ml<sup>-1</sup>) and *E. coli* ( $4.2 \times 10^1$  cfu ml<sup>-1</sup>) compared to the other ponds. Pond B contained significantly lower values than the other ponds in TBC ( $9.1 \times 10^2$  cfu ml<sup>-1</sup>) and coliform count ( $1.2 \times 10^1$  cfu ml<sup>-1</sup>). The *E. coli* count in ponds D, E, and G were significantly different ( $p < 0.01$ ) but in other ponds it was not detected.

The bacterial quality of silver carps harvested from the studied fish ponds is presented in Table 2. The results showed significant differences ( $p < 0.0001$ ) in the total bacterial counts and coliform counts of the fish harvested from the different ponds. Total and coliform bacterial quality of fishes is shown in the same table based on Duncan's test. The fish harvested from pond B had higher bacterial counts of total bacteria and coliform bacteria compared to the other ponds. Ponds D and C contained

significantly lower values than other ponds in TBC and coliform counts, respectively. Other bacterial counts including *E. coli* and *S. aureus* did not vary significantly ( $p > 0.05$ ). The bacteriological analysis of swab samples of silver carps showed 4 different genera of bacteria which included *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholera*, and *Listeria monocytogenes*. The culture results demonstrated that 35 (83.33%) fishes were found to be infected with Gram-positive bacteria and 23 (44.76%) fishes were infected with Gram-negative bacteria. Among them 21 (50%) fishes were infected with both groups of bacteria. The infection of *S. aureus* was the highest in 33 (78.57%) of the surveyed fishes, followed by *E. coli* in 20 (47.61%) fish sample. Moreover, 3 samples (7.14%) were found positive for *V. cholera* and 2 samples (4.76%) for *L. monocytogenes*. In all fishes harvested from warm-water ponds no evidence of *Salmonella* spp. and *V. parahaemolyticus* on surface of skin were observed.

The correlation coefficients between bacteriological counts of fish are shown in Table 3. TBC had a significant positive correlation with coliforms and *E. coli* counts, but correlation with *S. aureus* count was not significant ( $p > 0.05$ ). Coliform count had a significant positive correlation with *E. coli* count, but correlation with *S. aureus* count was not significant ( $p > 0.05$ ). *E. coli* had a significant positive correlation with all bacterial counts.

**Table 1: Means of bacterial counts (cfu/ml) obtained from water samples of seven studied ponds**

<i>Ponds</i>	<i>Total bacteria</i>	<i>Coliform bacteria</i>	<i>E. coli</i>
A	1.36×10 <sup>3</sup> *e	2.50×10 <sup>1</sup> *f	-
B	9.10×10 <sup>2</sup> *g	1.20×10 <sup>1</sup> *g	-
C	7.21×10 <sup>3</sup> *c	2.86×10 <sup>2</sup> *d	-
D	1.86×10 <sup>4</sup> *b	8.37×10 <sup>2</sup> *b	2.5×10 <sup>1</sup> *c
E	3.42×10 <sup>4</sup> *a	1.03×10 <sup>3</sup> *a	4.2×10 <sup>1</sup> *a
F	1.21×10 <sup>3</sup> *f	4.60×10 <sup>1</sup> *e	-
G	5.92×10 <sup>3</sup> *d	3.88×10 <sup>2</sup> *c	3.2×10 <sup>1</sup> *b

\*Means within a column, which are not preceded by a common superscript letter(s) are significantly different according to Duncan Multiple Range Test at  $P<0.05$  (Alpha 0.05)

- = Not detected

**Table 2: Means of bacterial counts (cfu/cm<sup>2</sup>) and detection of pathogenic bacteria obtained from skin swab samples of silver carps harvested from seven studied ponds**

<i>Ponds</i>	<i>Total bacteria</i>	<i>Coliform bacteria</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella</i>	<i>V. cholera</i>	<i>V. para.</i>	<i>L. mono.</i>
A	7.44×10 <sup>4</sup> *a	4.56×10 <sup>2</sup> *a	0.95	4.83	-	-	-	-
B	8.23×10 <sup>4</sup> *a	7.52×10 <sup>2</sup> *a	1.33	7.33	-	-	-	-
C	2.21×10 <sup>4</sup> *c	1.19×10 <sup>2</sup> *b	-	3.83	-	-	-	-
D	1.89×10 <sup>4</sup> *c	2.38×10 <sup>2</sup> *b	1.16	10.66	-	++	-	+
E	5.52×10 <sup>4</sup> *ab	4.00×10 <sup>2</sup> *a	3.42	25.33	-	+	-	+
F	3.95×10 <sup>4</sup> *b	3.96×10 <sup>2</sup> *a	0.92	4.83	-	-	-	-
G	5.72×10 <sup>4</sup> *ab	5.14×10 <sup>2</sup> *a	3.00	16.00	-	-	-	-

\*Means within a column, which are not preceded by a common superscript letter(s) are significantly different according to Duncan Multiple Range Test at  $P<0.05$  (Alpha 0.05)

- = Not detected; + = Detected in one sample; ++ = Detected in two samples

*V. para.* = *V. parahaemolyticus*; *L. mono.* = *L. monocytogenes*

**Table 3: Pearson correlation coefficients among the bacteriological counts obtained from skin swab samples of silver carps harvested from seven studied ponds**

	<i>Total bacteria</i>	<i>Coliforms</i>	<i>E. coli</i>	<i>S. aureus</i>
Total bacteria	1.00	0.70 **	0.40 *	0.13 <sup>NS</sup>
Coliforms	0.70 **	1.00	0.48 *	0.18 <sup>NS</sup>
<i>E. coli</i>	0.40 *	0.48 *	1.00	0.40 *
<i>S. aureus</i>	0.13 <sup>NS</sup>	0.18 <sup>NS</sup>	0.41 *	1.00

\*Correlation is significant at  $p<0.01$ ; \*\*Correlation is significant at  $p<0.0001$ ; NS = Non-Significant



## Discussion

This paper describes a study where different bacteria were tested to evaluate the bacterial load of warm-water fish ponds and contamination of silver carps which captured from these ponds.

In our study, a comparison of reference bacterial counts (total bacteria and coliforms) in pond water samples with the data provided by other authors (Ntengwe and Edema, 2008; Macedo et al., 2011) is indicative of low contamination of the ponds. When the bacterial counts in water are low, these microorganisms will be present in skin mucus and the digestive tract, but not in muscle tissue (El-Shafai et al., 2004). TBC in pond water samples were in the range of  $9.1 \times 10^2 - 3.42 \times 10^4$  cfu/ml which were lower than values recorded by Ntengwe and Edema (2008) in the order of  $10^6$  cfu/ml with the temperature of the ponds ranged from 17 to 25 °C. The obtained lower counts may be related to the lower temperature of the studied ponds which ranged from 8 to 12 °C. Also coliform counts were lower than that recorded by Macedo et al. (2011) who recorded  $9.4 \times 10^3 - 3 \times 10^8$  coliforms MPN/100ml in the rainy period.

Ponds A and B contained significantly lower values of coliforms and total bacteria in compared to other ponds. This could be related to the water resource of these ponds which were mainly supplied by underground waters. However, other ponds supplied by Sefidrood River introduced higher bacterial counts which could be due to the exposure of this source

to more contaminate than underground resource. For instance, water fowls like Geese and Swans that live around rivers, as Doyle and Ericson (2006) declared, can be a significant source of bacteria that elevate bacteria counts.

In this study, no *E. coli* detected in the analyzed water samples of ponds when their coliform counts were lower than  $2.86 \times 10^2$  cfu/ml, and this bacterium was only detected from those ponds with counts above  $3.88 \times 10^2$  cfu/ml. Also there was a positive correlation among the higher coliform counts and higher *E. coli* counts. In regards to the isolation of coliforms such as *E. coli* in some fish ponds, which is an indicator of fecal contamination (Feldhusen, 2000; Ekpo et al., 2010), results are in accordance with those obtained by Doyle and Ericson (2006) who reported that the ponds that harbor the fish could be the source of contaminates due to indiscriminate deposition of human, animal excreta and other environmental wastes into them. In addition to the potential risks of *E. coli* in fish skin for human consumption, being eaten raw or undercooked, the presence of *E. coli* in pond water can also put human beings at risk if they caught fish from the ponds and did not wash their hands before eating anything (Ntengwe and Edema, 2008).

Average of total bacterial counts of fish swab samples in studied ponds were ranged from  $1.89 \times 10^4$  to  $8.23 \times 10^4$  cfu/cm<sup>2</sup>. These are in accordance with those reported by Zmysłowska et al. (2002) that skin mucus usually contains  $10^2 - 10^7$  bacteria per cm<sup>2</sup> of skin.

Moreover, these values were found to be markedly lower than the recommended public health and safety standard value of between  $10^6 - 10^7$ , approved by Institute of Standards and Industrial Research of Iran (ISIRI). For fresh fish, the microbiological limit for human consumption proposed by the International Commission on Microbiological Specifications for Foods (ICMSF) (1986) is  $5 \times 10^5$  to  $10^7$  cfu/cm<sup>2</sup> in aerobic plate count analysis, which is also in line with our results.

About 90% of the fish swab samples investigated during the present study contained the coliform counts  $>10^2$ /cm<sup>2</sup> with a potential of causing health hazards as stated in study conducted by Jayasinghe and Rajakaruna (2005). However, most of the samples (57%) did not contain *E. coli* while 43% of 42 investigated samples of fish were with counts more than 2 and less than 6 per cm<sup>2</sup>. This is in disagreement with the report of Sivakami et al. (2008) who worked on microbial species in carps and noted that 50% of the organisms in carps were *E. coli* and *Pseudomonas aeruginosa*. According to ICMSF (1986) that recommended an 'm' value of 11 *E. coli* per cm<sup>2</sup> of fresh fish, results came in this study revealed that the quality of silver carps harvested from fish ponds is satisfactory. The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process (Novotny et al., 2004). Thus, as Empike et al. (2011) suggests, the public should be enlightened on the inherent danger that

may accompany handling fresh fish or consumption of improperly cooked fish.

The considerably high counts of coliforms and detecting *E. coli* recorded in this study indicates relative fecal contamination of fish which could be related to fecal contamination of studied fish ponds' water. This is in accordance with those obtained by Monzur-Hassan et al. (1994) who related presence of *E. coli* in fish to the fecal contamination of water with warm blooded animals. Also unhygienic handling of fish introduces the *E. coli* as secondary bacterial contaminants (*ibid.*).

Despite the lower values of coliforms and total bacteria obtained in water samples of ponds B and A, these values in fish swab samples collected from those ponds were higher than other ponds, relatively. This is in disagreement with the report of Ekpo et al. (2010), who suggested a strong relationship exist between fish bacterial load and that of the water ecosystem. This difference could be due to the unfavorable effect of the harvesting conditions on the bacterial contamination of fish. Therefore, precaution should be taken to prevent water contamination during harvesting as well as post harvest handling of fish (Emikpe et al., 2011). The microbial load of fish can also be improved through regular disinfection of catching gears or working equipment, and brief immersion of caught fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public (*ibid.*).



Nevertheless, the bacteriological results of other ponds did not show this kind of difference. Furthermore, water samples of pond E showed highest bacterial counts, and also fish samples of this pond revealed highest counts of *E. coli* and *S. aureus* and detection of some pathogenic bacteria, compared to other ponds. This suggests there is a relationship between bacterial contamination in fish and bacteriological quality of the harvest water which is in accordance with those obtained by other researchers (Jayasinghe and Rajakaruna, 2005; Reynisson et al. 2009; Ekpo et al., 2010). Besides, the co-existence of *E. coli* and the gram-positive *S. aureus* which cause food poisoning could have been transferred through contact with skin of handlers (Eze et al., 2011).

Significant differences were observed among the total bacterial counts and coliform counts in the fish harvested from the seven ponds. This difference could be due to the bacteriological quality of the water in which fish are harvested (CAC, 2010). However, silver carps harvested from the least contaminated pond (pond B) were the most infected fish in total bacteria, coliforms, and *E. coli* counts; but lower counts of *S. aureus* and pathogenic bacteria were observed in this pond. This finding is similar to those reported by Harnisz and Tucholski (2010) who noticed that the percentage content of aerobic and facultative bacteria decreased with an increase in total microbial counts. Moreover, our results showed that silver carps harvested from more contaminated ponds (Ponds E, D, and G) were the most

infected fish in *S. aureus* counts and the isolation of pathogenic bacteria. This is again in accordance with the findings of other researchers (Abou-Elela and Farag, 2004; El-Shafai et al., 2004; Harnisz and Tucholski, 2010) who noticed that the microbiological quality of fishes is directly related to the quality of the water in which they are cultivated and harvested.

Fish bacteriological results pointed out that *E. coli* counts had a positive correlation with *S. aureus* counts. This is in disagreement with report of Novotny et al. (2004) who declared such organism should not be present on fresh-caught fish. The presence of this fecal indicator bacterium in the fish could also be correlated with the presence of other pathogens. However, we did not find correlation between the values of coliforms and *E. coli* and isolation of *V.cholera* and *L. monocytogenes*. Similar to this finding, some authors (Feldhusen, 2000; Abou-Elela and Farag, 2004) have reported that no correlation was found between the presence of fecal coliforms and some pathogens e.g. *Vibrio* spp. In regards to Feldhusen (2000) and Novotny et al. (2004) reports, these isolated pathogens could be considered as potential food safety hazards of aquaculture products. Also silver carp could be considered as potential sources of infection with *L. monocytogenes* such as rainbow trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) which have been focused on during recent years (Novotny et al., 2004).

The fish harvested from pond D with total bacterial count of  $1.89 \times 10^4$

cfu/cm<sup>2</sup> which was the lowest count compared to the other ponds produced *V. cholera* and *L. monocytogenes*, while ponds A and B which had the highest TBC in fish did not produce these pathogens. These pathogens which categorized in indigenous bacteria group, when present in fresh cultured products, are usually found at fairly low levels, and where these products are adequately cooked, food safety hazards are insignificant (Feldhusen, 2000). However, these microorganisms could also enter seafood processing chain because of inadequate process control, poor standards of hygiene and sanitation in processing plants and post-production contamination during incorrect handling or storage (Claucas and Ward, 1996).

Also results revealed that, none of the samples investigated contained *Salmonella* spp. and *V. parahaemolyticus*. This could indicate that the fish harvested in the Guilan Province were free from these highly pathogenic bacteria. *Salmonella* can be present both in water, especially of contaminated ponds, and in the fresh fish from these areas, but low incidence and even absence is not uncommon (Feldhusen, 2000).

Many factors influence the microflora of finfish, the most important ones being water temperature, proximity of harvesting areas to human habitations, quantity and origin of food consumed by fish, and method of harvesting (CAC, 2010). Since the period of the investigation was in the cold season (winter) and the water temperature in the ponds was low (ranged 8 – 12°C), the effect of higher

temperatures in other seasons, which might cause the detection of more bacteria in ponds and fishes, could not be considered. Similar relationships were obtained by Zmysłowska et al. (2002); Jayasinghe and Rajakaruna (2005) who noticed that the lowest counts of the microorganisms determined were usually attained in winter, while higher counts were recorded in autumn and spring or in higher temperature zones. This observation can be related not only to the lower water temperature in winter, but also to the fact that silver carp primarily feed in the water column where bacterial concentrations are lower (Phong Lan et al., 2007).

Silver carps were found to harbor a number of pathogenic microorganisms in varying percentages on their skin. However, the quality of silver carps harvested from warm-water fish ponds in Guilan province, north of Iran were satisfactory in some bacterial indices, but detection of *E. coli*, *V. cholera*, and *L. monocytogenes* from fish samples could be considered as potential food safety hazards and initiate various fish-borne diseases in human if fish prepared improperly. The bacteriological qualities of these fishes could be substantially worse after unhygienic fish handling, cleaning and purchase in the local retail markets than that at harvest. Thus, it is recommended that a thorough surveillance of the microbiological status of fish be done for the safety of the ultimate consumers. Moreover, there is need for the Iranian fish farms and industry to improve on their fish farming management, such as, fish food

preparation, environmental sanitation and monitoring for quality fish yield.

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