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Detection of Microbiological Quality of Common Carp (*Cyprinus carpio*) Sold in Public Bazaar in Afyonkarahisar

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Abstract: In this study, a 100 carp fish that offered for sale in public bazaar in Afyonkarahisar were microbiologically examined in 2009 period (15 June to 15 July). In most of these samples, aerob total count was found between 10^7 and 10^8 kob mL^{-1}. Coliform count ranked 2nd after the aerob total count. Most of micrococcus and staphylococcus were found at 10^4-10^5 level. Although, the count of enterococcus was generally between 10^5 and 10^6, the count of enterobacter count was generally 10^6-10^7. Mold was not isolated from samples. Coliform bacteria level was 10^7-10^8 kob mL^{-1}. However, *E. coli* was under the detection level in most of the samples while it was 10^4-10^5 in the rest. On the other hand, Salmonella highly important for food infections was determined in 3% of the carp fish samples.

Key words: Fish, common carp, microbiological quality, Salmonella, food, infection

INTRODUCTION

Fish meat goes through certain chemical, physical and microbiological transformations during the period between fishing and consumption due to the effects of microorganisms, enzymes and other external factors. For this reason, it loses its freshness and quality.

Fish skin, gill and intestines of fishes fresh hunted from clean waters generally contain a high level of microorganisms however, they include only a limited number of microorganisms in muscles and thus, muscles are accepted sterile. On the other hand, microorganisms could be transmitted to muscles from gills, skins and intestines depending on the processes applied to fishes following the capture, storage temperature and duration and as a result, fish quality is deteriorated with the effects of microorganisms (Patir and Inanli, 2005).

It has been reported that the distribution of the bacteria types isolated from fishes is related to aquatic habitat of fishes and known to be affected by certain factors like the saltiness level and bacterial load of the habitat (Diler *et al*., 2000). Bacterial flora in fishes was determined to consist of mainly aerobic or facultative anaerobic and psychrophilic gram negative rods while the gram-positive bacteria is dominant in fishes of tropical regions (Cahill, 1990). As in all environments, pollution in water causes many problems for fish production. Especially, the contamination of natural and artificial lakes in Turkey with different pollutants negatively affects the health of people consuming the polluted water and fishes in these environments.

Expansion of culture fishery also increased the fish consumption and therefore, the bacterial disorders of fish origin have started to create problems. In the past, 15-20 bacteria species isolated from fishes were reported to demonstrate pathogenic effects however, nearly 70 bacteria species were isolated from fishes subsequently.

Common carp (*Cyprinus carpio*) is a fish species of economic importance in regions with temperate climate. It is found practically in every region of Turkey and accounts for an important portion of fish consumption (40%). The majority of the carp production in Turkey is carried out in Aegean, Central Anatolia and Southern Anatolia regions.

Common carp had an important place in the fishery development project in dam lakes of the General Directorate of State Hydraulic works and the mean carp yield in dam lakes was determined as 2.5 kg ha^{-1}. Fishery production in Afyonkarahisar other than culture fishery

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is 18.580 kg and common carp ranks first with 5.48%. Microbial flora of fish changes based on the microbial content of the water (Frazier and Westhoff, 1988). Especially in the developing countries, domestic and industrial wastes are discharged into rivers, lakes and seas without being adequately refined which cause serious environment problems.

In fact, waste waters containing plenty of organic matters create suitable cultures for the development microorganisms and the transportation of disease agents. This situation poses, a great danger for fishery products. On the other hand, fishes and other edible water creatures captured from clean waters contain little amount of bacteria. However, surface bacteria count is rapidly increased during and after the fishing. There are numerous studies reporting the presence of Salmonella in fishes and fish products in many Asian countries including Sri Lanka, Thailand, Taiwan and Indonesia (Fonseka, 1990; Rattagool *et al*., 1990; Chio and Chen, 1981; Retnowati *et al*., 1990).

Natural living environment of *Salmonella* sp. is gastrointestinal systems of reptiles, birds and mammals. For this reason, Salmonella species could easily spread to aquatic environment through fecal contamination. This situation explains the presence of Salmonella in fishes and fish products.

Fishes are transit transporters of Salmonella species. Salmonella species in waters are generally the *Salmonella serotypes* which are not adapted to host. Principally they cause septicemic infections, enterocolitis and local infections in human.

Contamination of water product origin in human is caused by the consumption of raw or undercooked fishes living in waters infected with Salmonella or later contaminated with Salmonella. Cross contaminations caused during the procurement, processing, packaging, transporting and storing stages and the breakage of cold chain are even more important factors in salmonellosis cases compared to primary contamination (Erol, 1999).

This study investigates, the microbiological quality of common carp (*Cyprinus carpio*) samples sold in the public market in Afyonkarahisar.

MATERIALS AND METHODS

Study material was composed of 100 common carp fishes sold in the public market in the Afyonkarahisar province in Turkey, 2009 (15 June to 15 July). Analyses were carried out after transporting the fishes in cold chain.

General microbiological analyses: Fishes were rinsed in sterile bags containing 225 mL Buffer peptone water (Oxoid CM0509); subsequently, plantation was made on cultures shown in Table 1 with drop plate method from the dilutions prepared up to 10^6 for the isolations of general aerobic mesophile organisms (ISO, 2003), Micrococcus/Staphylococcus (ISO, 1999), enterobacteria (ISO, 1993), enterococcus (Holth *et al*., 2000), yeast-mold (Anonymous, 1987) and Salmonella (ISO, 2002).

Coliform and *E. coli* isolations: Violet red bile lactose agar (Oxoid CM0107) was planted from suitable dilutions and the Petri dishes were left for incubation at 37°C for 24 h. Red-pink colored precipitation colonies were counted and evaluated. TBX (Tryptone Bile X-glucuronide- Oxoid, CM0945) culture was planted and left for incubation at 44.5°C for 24 h. Colonies suspected for reproducing green *E. coli* were subjected to Indol, Methyl red, Voges Proskauer and Citrate (IMVIC) tests. Colonies with positive IMVIC tests were considered as *E. coli* (ISO, 2001).

Salmonella isolation: Fishes were rinsed in sterile bags containing 225 mL Buffer peptonewater (Oxoid CM0509) and then the irrigation fluid was left for incubation at 37±1°C for 24 h. Following the incubation, 0.1 mL was planted to tubes containing 10 mL Rappaport-Vassiliadis

Table 1: Cultures used in the microbiological analysis and incubation conditions

		Incubation conditions		
Microorganisms	Cultures	Temperature (°C)	Duration	Aerobic/Anaerobic
Total aerobe mesophile general counts	Plate count agar (Oxoid CM0325)	30±1	24-72 h	Aerobic
Micrococci and staphylococci	Baird-parker agar base (Oxoid CM0275)	37	24-48 h	Aerobic
Enterobacteria	Violet red bile glucose agar (Oxoid CM0485)	35-37	24-48 h	Anaerobic
Coliform bacteria	Violet red bile lactose agar (Oxoid CM0107)	37	24-48 h	Aerobic
E.coli	TBX (tryptone bile X-glucuronide (Oxoid CM0945)	44.5	24 h	Aerobic
Enterococcus	Slanetz-bartley medium (Oxoid CM 377)	37	24-48 h	Aerobic
Yeast-mold	Rose bengal chloramphenicol agar (Oxoid CM 549, suppl. SR0078)	25	4-5 days	Aerobic
Salmonella	Buffer peptone water (Oxoid CM0509)	37	24 h	Aerobic
	Rappaport-vassiliadis (Oxoid CM 0866)	43	24 h	Aerobic
	Xysiloselysine deoxycholate (Oxoid CM0469)	37	24 h	Aerobic

Table 2: Result of biochemical test of *Salmonella* sp. isolates obtained from common carp (ISO, 2002)

Tests	Positive	Negative	*Salmonella* sp.
TSI	Yellow tip	Red tip	positive
TSI	Black color at bottom and gas formation	Not changing color	Positive
Urea broth	pink-red color	Not changing color	Negative
Lysine decarboxylase broth	Dark violet-purple	Yellow color	Positive
Methyle red	Diffuse red color	Diffuse yellow color	Positive
Voges-proskauer	Pink-red color	Not changing color	Negative
ONPG	changing color	Not changing color	Negative
Indole	Violet color on surface	Yellow color on surface	Negative
Salmonella polyvalent O	Agglutination present	Agglutination not present	Positive

medium (Oxoid CM 0866) of pre-enrichment fluids and the tubes were left for incubation at 43±1°C for 24 h. Subsequently, 1 lobe was planted to XLD (Xysiloselysine Deoxycholate, Oxoid CM0469 agar (CM 329, SR 87,117) during the selective enrichment. Petri dishes were left for incubation at 37±3°C for up to 48 h. Afterwards, five colonies suspected for pink colored and black centered Salmonella in XLD agar and pink coulered in BGA (modified) agar were selected. It was continued with nutrient agar (Oxoid CM0003). Colonies were identified by Gram staining and standard biochemical tests (triple sugar iron agar-CM 277; lysine iron agar-CM 381; urease test-CM 53; Simmons citrate-CM 155 and ONPG-disc-DD13 ONPG, MR-VP test-CM 0043) (ISO, 2002). Lastly, the lam agglutination test was applied with Salmonella polyvalent O and Vi antiserum for serologic test (O and H-Vi polyvalent antiserum, Difco 2264-47-2). Colonies with positive agglutination were accepted as Salmonella positive. Biochemical test results of Salmonella isolates were shown in Table 2.

RESULTS AND DISCUSSION

A total of 100 fishes sold in public market in Afyonkarahisar were microbiologically examined in the study and all analysis results were shown in Table 3. From this regard, fish samples were determined not compatible with the Fishery products regulations of Turkish food legislation in terms of general aerobic organisms, staphylococcus/micrococcus, coliform, *E. coli* and Salmonella contents of carp samples. As can be shown in Table 3 and 4, general aerobic organism counts of the analyzed carp samples changed between 10^7 and 10^8 cfu mL^{-1}. General aerobic organism count was followed by coliform bacteria. The majority of micrococcus and staphylococcus were determined between 10^6 and 10^7 cfu mL^{-1}, Enterobacteria count was generally between 10^6 and 10^7 cfu mL^{-} however, enterococcus was 10^5-10^6 cfu mL^{-1}.

Yeast count was determined as 10^5-10^7 cfu mL^{-1} for most samples and it was below the detection level in a limited number. No mold was isolated from samples. Coliform bacteria count was 10^7-10^8 cfu mL^{-1}. *E. coli* was below the detection level for many samples while it was found at 10^4-10^5 cfu mL level in the rest.

On the other hand, Salmonella important in terms of food infections was detected in three carp fish samples (3%). A total of 100 carp samples sold in public market in Afyonkarahisar were examined for index, indicator and pathogen microorganisms in the present study and the microbiological quality of the samples was found rather low.

Only 78% of the samples were convenient to Fishery products regulation of Turkish food legislation considering Total Aerobe Mesophile General Counts (TAMGC). As can be shown in Table 3, TAMGC of the analyzed carp samples changed between 10^4 and 10^9. These results were similar to the findings of studies performed on fished in Turkey. Diler *et al.* (2000) determined TAMGC in trout skin as 10^2-10^7 cfu g^{-1} while Bulduklu and Ozer found this count as 10^4-10^5 cfu g^{-1} in rainbow trout samples in their study.

Patir and Inanli (2005) investigated the micro-biological quality of horse-mackerel fishes stored at different temperatures and determined TAMGC as 3.50±2.57, 5.92±0.71 and 4.91±0.79 $\log_{10}$ cfu cm^{-2}. The total number of aerobic microorganism in fishes was reported as a significant criterion for human health and quality.

Total aerobic bacteria count in fish of high microbiological quality should be $<10^5$ cfu g^{-1} and the product is accepted spoiled if the bacteria count is $>10^8$ cfu g^{-1}. However, it has been reported that the initial microbial count in fishes is closely related to water quality and water temperature and it could change between 10^2-10^6 cfu g^{-1} in many different fish species (Acuff *et al.*, 1984; Savvaidis *et al.*, 2002).

Horsley reported lower aerobic bacteria count as 10^2-10^7 units cm^{-2} in Atlantic Salmon skin. Bacteria count in fishes of clean waters was determined as 10-100 units cm^{-2} while it was increased in fishes living in polluted regions or hot tropical waters (Huss, 1998). Horsley determined aerobic mesophile bacteria count as 10^2-10^4 cfu cm^{-2} while Gonzalez *et al.* (1999) reported this rate as 8.0×10^2 cfu g^{-1} for rainbow trout. The difference between the study findings was stated to be possibly caused by different fish species and measurement methods used in the studies.

Frazier and Westhoff (1988) stated that fishes demonstrated the characteristics of their habitats and bacterial flora could change among fishes produced in

Table 3: Result of microbiological analysis of common carp samples (%)

Samples	Microorganism level (kob g^{-1})	TAMGC (%)	Enterobacteria (%)	Coliform (%)	*E.coli* (%)	Enterococcus (%)	Mi/St (%)	Yeast (%)
Carp fish	$<2.0\times10^2$	-	11 (11)	9 (9)	88 (88)	25 (25)	8 (8)	18 (18)
	2.0×10^2-10^3	-	-	-	-	-	-	-
	10^3-10^4	-	-	1 (1)	3 (3)	-	4 (4)	4 (4)
	10^4-10^5	1 (1)	8 (8)	1 (1)	7 (7)	10 (10)	8 (8)	14 (14)
	10^5-10^6	3 (3)	24 (24)	12 (12)	1 (1)	40 (40)	21 (21)	38 (38)
	10^6-10^7	16 (16)	30 (30)	20 (20)	-	21 (21)	54 (54)	16 (16)
	10^7-10^8	78 (78)	27 (27)	55 (55)	1 (1)	4 (4)	5 (5)	10 (10)
n:100	10^8-10^9	2 (2)	-	2 (2)	-	-	-	-

TAMGC: Total Aerobe Mesophile General Counts; Mi/St: Micrococci and Staphylococci

Table 4: Numbers of logarithmical of microorganisms detected from common carp samples (log_{10})

Sample	Parameters	TAMGC (%)	Enterobacteria (%)	coliform (%)	*E. coli* (%)	Enterococcus (%)	Mi/St (%)	Yeast (%)
Carp fish	Min.	4.64	<2.30	<2.30	<2.30	<2.30	<2.30	<2.30
	Max.	8.47	7.95	8.27	7.07	7.38	7.44	7.68
(n:100)	Mean	7.30	6.73	6.73	4.23	6.07	6.27	6.41

different fish farms. A previous study established that there was no correlation between the total amount of anaerobic mesophile bacteria and the food quality however, this number reflected the hygienic quality (Aitken *et al*., 1982). Yeast-mold are hold responsible for the degradation of fishes. The majority of the samples were contaminated with yeast at 10^5-10^6 cfu mL^{-1} level. Similarly, Vural and Erkan determined yeast-mold level as 3.17 log_{10} cfu g^{-1} in fishes collected from Dicle river. This difference was possibly caused by the different fish species and measurement methods used in these studies.

Hygienic quality of the samples analyzed in the present study was determined rather low since there were 10^6-10^7 cfu mL^{-1} of enterobacteria in 30% of the samples, 10^8 cfu mL^{-1} of coliform bacteria and 10^5-10^7 cfu mL^{-1} of enterococcus. In the present study, coliform bacteria level was determined as 10^7-10^8 in 55% of the samples. Diler and Diler (1998) detected 1.5% of enterobacteria in 34 zander fishes. Diler *et al*. (2000) determined 6.17 and 6.96% enterobacteria in rainbow trout fishes grown in different farms. Vural and Erkan reported 5.24 log_{10} cfu g^{-1} of enterobacteria, 4.97 log_{10} cfu g^{-1} of coliform bacteria and 4.09 log_{10} cfu g^{-1} of *E. coli* in fishes collected from Dicle river. Similarly, Erdogrul and Bulbul (2006) reported to isolate *E. coli* in 24.4% of 41 atlantic tripletail fishes sold in the market.

Patir and Inanli (2005) determined minimum level of coliform bacteria isolated from the samples in their study as <1 log_{10} cfu cm^{-2}, maxium 6.16 log_{10} cfu cm^{-2} and mean as 4.01±1.75 log_{10} cfu cm^{-2}. The number of coliform bacteria in fishes accepted as fecal contamination indicator was generally reported as 2.0×10^2, 2.5×10^2 and 1.6×10^3 cfu g^{-1} changing based on the fish species (Jay, 1996; Shewan, 1971). Patir and Inanli (2005) determined coliform bacteri in horse-mackerel fishes as 4.01±1.75 log_{10} cfu cm^{-2} on average and while they did not found any *E. coli*.

Coliform bacteria were not detected in the skins and muscles of fishes captured in clean waters. Absence of fecal coliform bacteria known as indicator microorganisms, implied that either the fishes were captured in waters contaminated with fecal bacteria or they were contaminated during the applications (transportation or processing) following capture (Patir and Inanli, 2005). Vural and Erkan found staphylococcus-micrococcus at 4.47 log_{10} kob g^{-1} level in the fishes captured from Dicle river. Patir and Inanli (2005) reported to detect Staphylococcus-Micrococcus in 30 horse-mackerel fishes at 3.90±0.57 log_{10} cfu cm^{-2} level while no *Staphylococcus aureus* was isolated from the samples. It was stated that Staphylococcus <100 g^{-1} indicated the human-originated contamination . The initial bacteria population in fresh sea products of in Mexico was reported to be composed of Micrococcus, Coryneform and other gram-positive bacteria in general. Similarly, statistical data showed the presence of differences among the samples considering microorganisms of this group and there was no uniformity.

Salmonella microorganism is rarely seen in fishes. However, it could reproduce upon the contamination from environment during the processing stage of fishes. Another contamination possibility is that fishes could be infected with salmonella in polluted sea and lake waters. Previous studies examining the presence of Salmonella reported that different fish species could be contaminated with *Salmonella* sp. (Erdogrul and Bulbul, 2006). Salmonella prevalence has been reported to demonstrate wide variations in fishes, mussles and other sea products in different parts of the world. Studies implemented in Taiwan, Sri Lanka, Thailand, India, Vietnam, Japan, Europe and America stated that Salmonella prevalence changed between 1.3 and 24.5% (Heinitz *et al*., 2000; Iyer and Shrivastava, 1989; Kumar *et al*., 2003; Phan *et al*., 2005, Rattagool *et al*., 1990; Saheki *et al*., 1989). In addition,

there are also many studies reporting Salmonella rate between 0 and 28.2% (Belchior and Pucci, 2000; D'Aoust *et al*., 1980; Hatha and Lakshmanaperumalsamy, 1997; Pao *et al*., 2008; Youssef *et al*., 1992).

In a study, performed by FDA between 1990 and 1998, Salmonella incidence was found as 7.2% in 11.312 imported sea products and 1.3% in 768 domestic sea products (Heinitz *et al*., 2000). In another study carried out in Japan, *Salmonella* sp. was detected as 21% in culture eel pools (Saheki *et al*., 1989). Similarly, Salmonella incidence in culture catfish waters was determined as 5% (Wyatt *et al*., 1979). There are many studies in India reporting Salmonella incidence in sea products.

Varma detected Salmonella prevalence as 7.46% in frozen shrimp shells and shrimps internal organs. Iyer and Shrivastava (1989) reported Salmonella incidence as 12% in eel and shrimps without internal organs, 10% in shrimps sold without head, 14% in shrimps without shell and internal organs, 17% in lobster, 14% in cuttlefish, 25% in catfish and 20% in seer fish (*Cybium commersonii*). The highest Salmonella incidence in sea products was determined in Central Pacific and African countries while it was lower in Europe and United States of America. Salmonella presence in sea products was also reported for Vietnam, India, Sri Lanka and Japan (Elizabeth *et al*., 2008). In the present study, salmonella was isolated from three carp fish samples.

Different prevalence was attributed to the different origins of sea and freshwater fishes. From this regard, Salmonella prevalence was found higher in tropical Asia-Pacific, African and European countries and Russia while it was lower in North America (Heinitz *et al*., 2000). It was reported that 24.5% of shrimps in Vietnam were contaminated with *Salmonella weltevreden* and *Salmonella tennessee*. *Salmonella* species are among the most common pathogens causing enteritis. Diarrhea is one of the most important problems of developing countries and found related with unsuitable sanitation conditions (Phan *et al*., 2005).

The main source of bacteria was established by the previous studies as the contamination following the capture. This contamination was caused by fish container, factory conditions and distribution system (Heinitz *et al*., 2000; Kimura *et al*., 2001).

Microbial contamination was reported to be depended on water, fishing conditions and unsuitable processing, distribution and storage applications following the capture of fishes. Cross contamination between raw and cooked fishes, faulty heat applications and the consumption of raw fishes were stated to contribute to the poisoning cases of sea product origins (Fletcher *et al*., 1998). Water and fishery product intoxications could be prevented by providing convenient conditions in all the processing stages of fishes, effectively applying cooling system and controlling the storage temperature (Craven *et al*., 2001; Hayes, 1987; Sabroe and Blok, 1998).

CONCLUSION

In this study, it was determined that hygienic quality of carp fish samples collected from public market was rather low and the majority of the samples were not compatible with the Fishery products regulation of Turkish food legislation. The presence of staphylococcus, *E. coli* and Salmonella in fishes which are pathogen microorganisms significant for food infection and intoxications are highly important for public health (Razem and Katusin-Razem, 1994). In the present study, it was highly noteworthy that *E .coli* and Salmonella were detected at 7 and 3% levels in the analyzed carp fishes. Fishing conditions, storage type after capture, unsuitable processing and distribution conditions are effective in fish intoxications.

Cross contaminations between raw and cooked fishes, faulty heating applications and consumption of raw fishes also contribute to poisoning cases. Water and fishery product intoxications could be prevented by providing convenient conditions in all the processing stages of fishes, effectively applying cooling system and controlling the storage temperature.

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