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Prevalence, serotypes distribution and characterization of Salmonella in common carp (Cyprinus Carpio), Afyonkarahisar Province, Turkey

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Abstract

This study was performed to investigate the prevalence of Salmonella spp. in 100 free freshwater common carp samples using the classic culture technique. For the confirmation of the isolates at molecular levels, the *invA* gene was detected. Serotyping of the isolates was also detected. The presence of invA, class 1 (Cls1) integrons, and integrase (Int1) genes was demonstrated by PCR assay; and the resistance of the Salmonella spp. strains to antibiotics was determined by disk diffusion test. Salmonella were detected in 3% (n=7 isolates) of the samples. Three different serotypes were detected; S. Panama, Enteritidis and Quinella. S. Panama is predominant. The invA was detected in the isolates. To detect antibiotic susceptibility, 14 different antibiotics were evaluated using the disc diffusion method. The isolates were evaluated for β-lactamase production. All isolates were resistant to erythromycin, penicillin, oxacillin, vancomycin and clindamycin. Multiple resistances were found in all isolates. S. Enteritidis and one S. Panama were the most resistant serovars. The Cls1 were detected in all isolates. In contrast, int1 were detected in 57.14%. In conclusion, the prevalence of Salmonella in the common carp can pose a risk to the public due to foodborne salmonellosis, multiple antibiotic resistance properties and the potential transfer of drug resistance genes to other members of the Enterobacteriaceae and humans via Cls1 integron.

Keywords: Salmonella, Common carp, Serotypes, Antibiotic resistance, Integron/integrase

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Introduction

Salmonellosis and the emergence of multidrug resistant (MDR) Salmonella have become an increasing concern worldwide. In the United States, it is estimated that 1.2 million salmonellosis occurs each year, with more than 23,000 hospitalizations and 450 deaths (Center of Disease Control, 2013). In the European Union countries, in 2007, there were 130 outbreaks associated with fish and fishery products, three of which were Salmonella linked outbreaks (FAO, 2011). The presence of Salmonella serovars in fish, shellfish and other seafood has been reported and the prevalence of Salmonella has been detected in the range of 0.0-28.2% (BGVV, 1996; Broughton and Walker, 2009; Siriken et al., 2010; Raufu et al., 2014).

Antimicrobial resistance (AR) and, in particular, multidrug resistance (MDR) is a major public health concern, worldwide and locally, due to the circulation of persistent resistant bacterial strains in the environment and the possible contamination of water and particularly foods of animal origin (Sorum and L'Abee Lund, 2002). The main resistance stems are due to the use antimicrobials in human veterinary medicine, animal husbandry, agriculture and aquaculture practices. World Health Organization (WHO, 2002) reported that about half of the total amount of antimicrobials produced globally is used in food animals. It is also reported that, particularly in Asia, the use of various antimicrobials are licensed and used in fish and shrimp production (Angulo et al., 2004).

The reservoir of antibiotics resistance between different can interact ecological systems and the potential transfer of resistant bacteria or resistant genes from animals to humans may occur through the food chain (Witte, 2002). Also, mobile genetic elements in Salmonella strains, such as plasmids, transposons and integrons play an important role in the evolution and dissemination of multidrug resistance (Boyd et al., 2002). Integrons are one of the genetic elements involved in the acquisition of antibiotic resistance. It has been reported that MDR among Salmonella serovars is often associated with the presence of class 1 integrons carrying multiple resistance genes (Antunes et al., 2004; Khan et al., 2006). The presence of integrase (*Int1*) is known to be an essential part of all integrons. A functional integrase is necessary to continue the catalytic process for the insertion/deletion of gene cassettes (Hanau-Bercot et al., 2002), and its presence is potentially indicative of strains capable recruiting antibiotic resistance genes (Di Conza and Gutkind, 2010).

The *invA* gene is specific for *Salmonella* spp. (Chen and Grifiths, 2001), thus, it represents a suitable PCR target with potential diagnostic applications (Rahn *et al.*, 1992). In addition, it is also a virulence gene. *InvA* gene, a member of genetic locus, allows *Salmonella* to enter cultured epithelial cells (Galán *et al.*, 1992).

Common carp (*Cyprinus carpio*) is a widespread freshwater fish species and occurs in several non-native areas worldwide. It is the most widely

cultured Cyprinid in Central and Eastern Europe, the Caucasus Central Asia (FAO, 2011). It has great economic importance in temperate climate regions of the world. Turkey also represents one of the regions for evaluating common carp growth. The species is bred in almost all parts of Turkey, especially the Aegean, Internal and South regions. The total catch of freshwater products in 2013 was 35,074 Turkey. According tons in TURKSAT (2013) data, the common carp production in Afyonkarahisar (inland province of Turkey) was 75 tons in 2013.

Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Most people suffering from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Epidemiological studies demand for the proper identification of the serotype and determination of the antimicrobial resistance/susceptibility profile. Therefore, the aim of this study was to investigate the presence of Salmonella, to determine the invA gene (genus specific), serotyping, antibiotic resistance (AR) profile and presence of Cls1 and integrase genes of the Salmonella isolates from common carp fish samples.

Material and methods

Sample collection

A total of 100 wild (free fresh water) common carp samples were used to investigate the presence of *Salmonella*

spp., to determine the *invA* gene (genus specific), serotyping, antibiotic resistance (AR) profile and presence of Cls1 and integrase genes of the isolates. The free fresh fish (caught in dam lakes) were randomly collected from markets in the Afyonkarahisar Province in Turkey, 2009. All of the samples were transported under refrigerated conditions and then analyzed promptly.

Salmonella species isolation and identification

The isolation of Salmonella species was carried out in two enrichment steps; the whole carp (350-400 g) was aseptically transferred into a sterile polyethylene stomacher bag and 225 ml buffered peptone water (BPW, CM 0509) was added, homogenized for 1-2 min, removed aseptically, and the incubated at 37°C for 18 ± 2 Following the incubation, 0.1 ml of each BPW incubated was transferred to 10 ml of Rappaport Vassiliadis (RV) enrichment broth (CM 0866), and incubated again at 41.5 °C for 24 h. Afterwards, the culture was streaked onto both Xylose Lysine Deoxycholate CM 0469) (XLD, and Modified Brilliant Green Agar plates (BGA) (CM 329, SR 87,117). Then, the plates were incubated for up to 48 h at 37°C (ISO, 2002). After the incubation, up to five colonies suspected of being Salmonella spp. from each plate were selected and sub-cultured onto Nutrient Agar (CM 0003). The colonies were identified by staining and standard Gram biochemical tests (triple sugar iron agar-CM 277; lysine iron agar-CM 381; urease test-CM 53; Simmons citrateCM 155, and ONPG-disc- DD13 ONPG, MR-VP test – CM 0043). All the media used were from Oxoid Ltd., Basingstoke, United Kingdom. Confirmation was made with *Salmonella* antiserum (O and H-Vi polyvalent antiserum, Difco 2264-47-2) (Flowers *et al.*, 1992).

Serotyping of the Salmonella spp.

Salmonella serotyping was carried out at T.R. Minister of Health, The Refik Saydam Hıfzıssıha Center, The Management of Epidemic Diseases Research, The Enteric Pathogen Laboratory, Ankara, Turkey.

Detection of genus-specific invA gene of Salmonella spp.

The template DNA was initially obtained using the boiling method. Following this, the supernatant containing the DNA was transferred into Dnase/Rnase-free microcentrifuge tubes and stored at -20 °C for use as the template DNA.

For detection of genus-specific *invA* gene of *Salmonella* spp., a single target PCR assay was used according to Salehi *et al.* (2005). *S.* Typhimurium (ATCC 14028) as a positive control. During the procedures, a gradient thermocycler (Bio Rad-MJ Mini-PTC-1148, Singapore) was used.

Ampflication of bacterial DNA was performed with 50 μl volumes containing 5 µl of extracted DNA used as a template; 1X **PCR** Buffer (Fermentas), 1.5 mM $MgCl_2$ (Fermentas), 200 μΜ of each deoxynucleoside triphosphate (dNTP) (Fermentas), 1.25 U Tag **DNA** Polymerase (Fermentas) and $0.5~\mu M$ each of primers. The conditions for the PCR were 94 °C for 1 min for initial denaturation of DNA within the sample, followed by 35 cycles of 94 °C for 1 min (denaturation), 64 °C for 30 sec (primer annealing), 72 °C for 30 sec (DNA synthesis), and 72 °C for 7 min (final extension). The samples were electrophoresed for 90 min at 100 V. Amplified DNA fragments of specific sizes were located by UV fluorescence after being stained with ethidium bromide.

Detection of Class 1 Integron and of integrase gene

The single target assays for each gene and nucleotide sequence (*invA*, *Cls1* and *IntI1*) of the primers were applied according to Bass *et al.* (1999) (Table 1).

For the class 1 integron gene detection, ampflication of bacterial DNA was performed with 50 ul volumes containing 5 µl of extracted DNA used as a template; 1X PCR Buffer (Fermentas), 3.25 mM MgCl₂ (Fermentas), 200 μM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 2.50 U Taq Polymerase (Fermentas) and 0.22 μM each of primers.

For the IntI(integrase) gene detection, ampflication of bacterial DNA was performed with 50 µl volumes containing 5 µl of extracted DNA used as a template; 1X PCR Buffer (Fermentas), 2.5 mM MgCl₂ (Fermentas), 200 μM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 1.25 U Tag **DNA**

Polymerase (Fermentas) and 0.22 μM each of primers.

Both class 1 integron and integrase gene detection amplication programs were the same. The conditions for the PCR were 94 °C for 5 min for initial denaturation of DNA within the sample,

followed by 30 cycles of 94 °C for 1 min (denaturation), 55 °C for 1.5 min (primer annealing), 72 °C for 1 min (DNA synthesis), and 72 °C for 1 min (final extension).

Table 1: Nucleotide sequences of the primers.

Amplified	Oligonucleotid sequence	Products (bp)	Reference	
invA	F 5 ¹ - GTG AAA TTA TCG CCA CGT TCG GGC AA -3 ¹ R 5 ¹ - TCA TCG CAG CGT CAA AGG AAC -3 ¹	284	Bass et al. (1999)	
ClsI	5' GGC ATC CAA GCA GCA AG 3' 5' AAG CAG ACT TGA CCT GA 3'	Varied	Bass et al.(1999)	
IntI1	5' CCT CCC GCA CGA TGA TC 3' 5' TCC ACG CAC TGT CAG GC 3'	280	Bass <i>et al.</i> (1999)	

Antibiotic susceptibility testing

isolates were tested against erythromycin (15µg, Oxoid), neomycin $(10\mu g,$ Oxoid), gentamicin $(10\mu g,$ Oxoid), tetracycline (10µg, Oxoid), cefotaxime (30µg Oxoid,), amikacin $(30\mu g,$ Oxoid.). cefalotine $(30\mu g,$ Oxoid), penicillin $(10\mu g,$ Oxoid), oxacillin (1µg, Oxoid), vancomycin (30µg, Oxoid), streptomycin (10µg, Oxoid), ampicillin (10µg, Oxoid). chloramphenicol (30µg, Oxoid) and clindamycin (2µg, Oxoid) by using the Kirby-Bauer disc diffusion method. The resistance levels were defined described by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2010) and indicated as susceptible (S) or resistant (R).

Detection of β -lactamase

For this purpose, the chromogenic cephalosporin method using commercially prepared nitrocephin touch (Oxoid. impregnated sticks BR0066A, Basingstoke, Hampshire, England) were used according to the manufacturer's instructions. In brief, a representative pure colony from the growth medium was selected. The stick was then rotated to pick up a small mass of pure cells and was observed for 10 minutes. A colour change from yellow to pink-red indicated positive βlactamaselactamase producing organisms.

Results

In the present study, a total of 7 *Salmonella* isolates were isolated from three (3%) out of the 100 samples by using the cultural technique and PCR (Fig. 1).

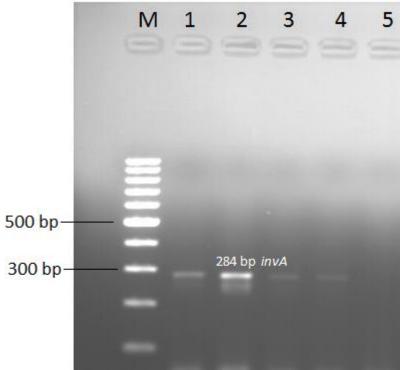


Figure 1: Determination of the *invA* gene in the *Salmonella* strains isolated from Common carp samples using PCR technique. M: Marker; 1, 3 and 4 Lanes; *Salmonella* spp. isolated from Common carp samples, 2 Lane; *S. enteritidis* ATCC 13076 (positive control), 5 Lane; negative isolate.

According to serotyping results, three different serotypes were detected in seven isolates, one of which was *S. enteritidis*, five of which were *S. panama* and one of which was *S. quinella*. *S. enteritidis* and *S. panama* isolates were obtained from one of the three samples. Three *S. panama* strains were determined; the other one sample and the last two *S. panama* and *S. quinella* strains were also obtained from one of the samples.

All of the isolates were resistant to erythromycin, penicillin, oxacillin,

vancomycin and clindamycin. A higher frequency of resistance to neomycin (42.85%) and streptomycin (57.14%), and a low frequency of resistance to gentamicin (14.28%) were found in the isolates. All of the isolates were susceptible to tetracycline, cefotaxime, amikacin, cephalothin, ampicillin and chloramphenicol (Table 2). B-lactamase production was not detected in the isolates.

Table 2: Antibiotics resistance, Class 1 integron and integrase profile of Salmonella serovars obtained from 3 samples.

No	Antibiotics Resistance Number	%	S.enteritidis (1.a)	S.panama (1.b)	S.panama (2.a)	S.panama (2.b)	S.panama (2.c)	S.panama (3.a)	S.quinella (3.b)
1	Erythromycin (15	100	R	R	R	R	R	R	R
2	μg) (n=7) Neomycin (10 μg)	42.85	S	R	S	S	S	R	R
3	(n=3) Gentamicin (10 μg) (n=1)	14.28	R	S	S	S	S	S	S
4	Tetracycline (10 μg) (n=0)	0.0	S	S	S	S	S	S	S
5	Cefotaxime (30 µg) (n=0)	0.0	S	S	S	S	S	S	S
6	Amikacin (30 μg) (n=0)	0.0	S	S	S	S	S	S	S
7	Cephalothin (30 μg) (n=0)	0.0	S	S	S	S	S	S	S
8	Penicillin G (10 µg) (n=7)	100	R	R	R	R	R	R	R
9	Oxacillin (1 µg) (n=7)	100	R	R	R	R	R	R	R
10	Vancomycin (30 µg) (n=7)	100	R	R	R	R	R	R	R
11	Streptomycin (10 µg) (n=4)	57.14	R	S	R	R	S	R	S
12	Ampicillin (10 μg) (n=0)	0.0	S	S	S	S	S	S	S
13	Chloramphenicol (30 μg) (n=0)	0.0	S	S	S	S	S	S	S
14	Clindamycin (2 µg) (n=7)	100	R	R	R	R	R	R	R
	Cls1 integrons (n=7)	100	1000bp	1000 bp 1500 bp	1000 bp	1000 bp	1000 bp 1500 bp	1000 bp 1500 bp	1000 bp
	Integrase (n=4)	57.14	Present	-	Present	-	-	Present	Present

*S.enteritidis (1.a) and S. Panama (1.b) isolates were obtained from one of the three samples. Three S. Panama (2.a,b,c) strains were determined in the other one same sample, and the last two S. Panama (3.a) and S. Quinella (3.b) strains were also obtained from one of the same samples.

In the present study, Cls1 integrons were detected in all of the isolates (Fig. 2). There were two kinds of integrons present: 1000 bp and 1500 bp together carried isolates and 1000 bp present isolate alone. Two variable regions carrying isolates were the three *S*. Panama serotypes. Single region

carrying isolates were the *S. enteritidis*, two *S. panama* and *S. quinella* serotypes. In contrast to the integrons, the total *intI1* carrying isolate number was four, and it belonged to *Salmonella enteritidis*, two Panama and Quinella serotypes (Fig. 3).

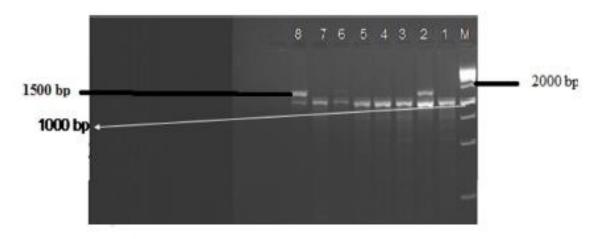


Figure 2: Determination of the Class 1 integron in the Salmonella strain sisolated from Common carp samples. Lane M: Marker, Lane 2,6 and 8: 1000 And 1500 bp integrons (two different variable regions-three S. Panama isolates); Lane 1: Positive control (chicken carcass origin Salmonella isolate); Lane 3,4,5 and 7: 1000 bp integron isolates (S. enteritis, two S. panama and S. quinella serotypes, respectively).

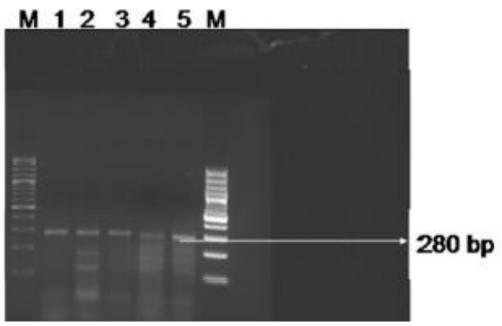


Figure 3: The determination of the integrase (*Inti*) gene in the *Salmonella* strains isolated from Common Carp samples. M: Marker, Lanes 1: Positive control (chicken carcass origin) *Salmonella* isolate), Lane 2, 3, 4 and 5 positive *Salmonella* isolates (*S. enteritis*, *S. panama* and *S. quinella*, respectively).

The integron carrying isolates were resistant to at least five antibiotics: erytromycin, penicillin, oxacillin, vancomycin and clindamycin (Table 2). Among the serovars, *S.* Enteritidis and two *S.* Panama isolates were also resistant to streptomycin, and *S.* Quinella serovar was resistant to

neomycin besides the five antibiotics. However, the remaining three isolates without integrons and integrase were also resistant at least to the same five antibiotics.

Discussion

Several studies on Salmonella in fish and fish products have been conducted in various countries. For instance, Broughton and Walker (2009) reported that the prevalence of Salmonella is 5% (n=100) live freshwater-farmed finfish in China. Another study reported that the incidence of Salmonella in raw import fish was 12.2% in the USA (Heinitz et al., 2000). There have also been manv studies limited Salmonella in fish and contamination results range from 0.0 to 28.2% 1996: (BGVV. Hatha and Lakshmanaperumalsamy, 1997; Kumar et al., 2003; Pao et al., 2008; Raufu et al., 2014). The results of the present study and of others mentioned above indicate that the prevalence Salmonella in freshwater fish, farmraised fish and other seafood samples has showed wide variation around the world. This variation may depend on marine or water contamination levels due to rainfall events, a variety of animal waste, fertilization of ponds, contaminated feeds. contaminated farm source water or primary processing etc. For instance, Kumar et al. (2003) reported that, the presence of Salmonella in seafood was found to be quite variable, although samples were collected from 234 different locations, they were collected from similar environments.

In Turkey specifically, to date, there has been very limited data generated on *Salmonella* spp. in fresh marine fish, to the knowledge of the authors of the present study. One of them, Siriken *et al.* (2010) reported that *Salmonella*

strains were not detected in 150 fresh seafood (fish. mussel and anchovy) samples obtained from the Black Sea, Turkey. In the present study, Salmonella however, strains isolated from common carp (3%)caught in dam lakes. The difference between the results of the two studies may depend on the origin of the fish (open sea vs. Dam Lake). Likewise, Huss and Gram (2004) reported that Salmonella can multiply and survive in the estuarine environments and tropical freshwater environments for weeks although open marine waters are free from Salmonella. The results of the two studies that are mentioned supported this opinion.

Serotyping is the most widely used phenotyping method for epidemiological investigation of Salmonella (Buchanan and Gibbons, 1974). There are several studies with regard to Salmonella isolates related to fish or seafood. One of them by Zhao et al. (2003) determined 82 serotypes and 187 isolates associated with 4072 imported seafood samples. The other studies from around the world reported that S. Weltevreden is the most frequently detected serotype in seafood samples (Heinitz et al., 2000; Zhao et al., 2003; Bhowmick et al., 2012). FAO (2010) also reported that the most common serotype encountered was S. Worthington followed by S. Weltevreden, and the diversity of serovars associated with fish and fisherv products was highest Southeast Asia and next highest in South America. In this study, 71.42 % of Salmonella isolates belonged to the serotype S. Panama. Beside S. panama, S. enteritidis (14.28%) and S. quinella (14.28%) also identified as Salmonella serotypes. According to all of these findings from around the world, it seems that S. Weltevreden is the most common serotype in fish and seafood, which is in contrast to the present study. The biggest difference among severity and treatment methods is between enteric fever salmonellae nontyphoid salmonellae. Hence, a combination of factors specific to each serovar including the presence of plasmid virulence genes, surface cell structure, flagellin, and pathogenity islands is involved in severity of salmonellosis (Ginocchio et al., 1997). Among Salmonella serotypes, serovar Enteritidis has been the most frequently isolated followed by Typhimurium, Newport, Heidelberg, and Montevideo. addition. S. Typhimurium, S. Enteritidis, S. Newport, S. Heidelberg serovars are closely related to foodborne outbreaks (Rodriguez et al., 2006; Fangtham and Wilde, 2008). In the study, S. Enteritidis was detected in only one isolate and in the other study based on fish samples mentioned above. the important non-tyhoid serotypes for human was not detected. In the present study, the antibiotic resistance was investigated in Salmonella isolates by using antibiotics from eight different antibiotic classes (aminoglycosides, tetracyclines, vancomisin, lincosomides, macrolides, phenicol, cephalosporins, ß-lactamase stable penicillin). **B-lactamase** production was determined, too. In this respect, the antimicrobial susceptibility test results of the present study showed that all of the isolates were sensitive to 6 out of 14 antibiotics tested. All of the isolates were also not capable of Blactamase production. In contrast, all of the isolates were resistant to at least 5 antibiotics. One of them was of the macrolide group (erythromycin), two of which were of the \(\beta \)-lactamase stable penicillin group (ampicillin and oxacillin and penicillin), one of which the lincosamides (clindamycin) and the last one was vancomycin antibiotics. Generally, clindamycin and vancomycin are not used against Gram negative infections. Therefore, the two antibiotics are not important to salmonellosis treatment. Almost half of the Salmonella isolates were also resistant to neomycin and streptomycin (aminoglycoside group) antibiotics.

The occurrence of Salmonella serovars resistant quinolones, to fluoroquinones, and third generation cephalosporins which are medically significant treatments has increased. Among Salmonella serotypes, although S. Enteritidis is highly prevalent in human infections: it has lower antimicrobial resistance compared to other serovars. According to a NARMS (2010), the serovars with greater resistance to antimicrobials are Typhimurium specific to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole. and tetracycline (ACSSuT), as well as Enteritidis with resistance to naldixic acid. Serovars Newport, Heidelberg, Dublin were also shown to be resistant to various antimicrobial groups. In

terms of multidrug resistance the most prevalent serovars of epidemiological importance Typhimurium, are Heidelberg, Dublin, and Paratyphi B (Andino and Hanning, Antimicrobial resistance in Salmonella can be associated with horizontal transference of antibiotic resistant genes characteristically found on mobile genetic elements among Salmonella strains or by clonal spread antimicrobial drug resistant serovars particularly effective are worldwide dissemination (Mather et al., 2013). It is believed that horizontal transmission of virulence genes in multidrug resistant Salmonella strains can increase virulence and invasiveness cause higher mortality rates and compared to susceptible Salmonella (Angulo and Mølbak, 2005; Han et al., 2011). In terms of multidrug resistance, most prevalent serovars of importance epidemiological are Typhimurium, Heidelberg, Dublin. Paratyphi B (Andino and Hanning, 2015).

There have been several studies with regard to the antibiotic resistant profile of Salmonella. One of them was reported from China by Broughton and Walker (2009). They reported that all five Salmonella strains isolated from 100 live freshwater-farmed finfish were susceptible to neomycin, cefotaxime, and cefepime and resistant erythromycin and penicillin. Zhao et al. (2003) reported that 8% (15/187) of their Salmonella isolates were resistant to at least one antimicrobial, and 2.7% (5/187) were resistant to three or more antimicrobials. Khan et al. (2006)

reported that a total of 105 S. enterica strains were isolated from imported seafood from 20 countries in the US from 2000 to 2005. Among these S. enterica isolates, strains two **Bareily** and Oslo) (serovars that originated from two different countries (Vietnam and India) were resistant to trimethoprim/sulfamethoxazole,

sulfisoxazole, ampicillin, tetracycline and chloramphenicol. In another study, reported that three Typhimurium and three S. Weltevreden strains were resistant to an array of antibiotics which include eight ampicillin, carbenicillin, doxycycline, kanamicin, nalidixic acid, sulfafurazole, tetracycline and trimethoprim. These results show wide variations. These antibiotics resistant properties Salmonella isolated seafood samples us that the antibiotics show accumulated in the aquatic environment. which provides the antibiotic stress for required the emergence of more and more antibiotic resistant phenotypes of Salmonella due to the indiscriminate use. It has also reported that MDR among Salmonella serotypes and other clinical isolates is often associated with the presence of Cls1 integrons (Antunes et al., 2004; Wannaprasata et al., 2011). The results of the present study also showed that integrase was present in 57.14% of the Salmonella isolates, which possessed 1 kbp (75%) size alone and 1kb together with 1.5 kb (25%) sizes of Cls1 integron. Similar results reported from Germany, UK, Japan, China, USA, Ethiopia and Iran showed that the prevalence of Cls1 integrons among the MDR Salmonella seovars isolated from various types of animal origin food and humans were found between 11 and 65% (Randall et al., 2004; Zhang et al., 2004; Ahmed et al., 2005; Miko et al., 2005; Meng et al., 2011; Firoozeh et al., 2012). However, there are a few studies with regard to Cls1 integron containing Salmonella strains isolated from fish and other seafood (Heinitz et al., 2000; Khan et al., 2006). In the present study, on the other hand, we also found that the three S. Panama serotypes did not carry int1. However, these three S. Panama isolates were also MDR. The reason may depend on other factors such as chromosomal mutation or the presence of different class integrons or other genetic elements such as transposons and plasmids.

For the detection/confirmation of Salmonella isolates. particularly link seafood to isolates, various Salmonella species-specific gene regions were determined using PCR assay. These are oriC, ompC, random fragment, invA, invE or hns etc. However, there are variations in the detection limits or accuracy among the genes (Shabarinath et al., 2007). For instance, Shabarinanth et al. (2007) reported that among hns, invA and invE genes, hns detected Salmonella was significantly higher in number among the samples. In the present study, the invA gene was detected in 7 out of 8 Salmonella isolates. After the PCR assay, we searched to find which bacteria the isolate belongs to. It was confirmed as Citrobocter spp. There are several virulence genes (invA, phoP,

hilA, spvC and spvR) reported among Salmonella species (Bakshi et al., 2003). Among these virulent genes, the invA gene codes a Type III protein secretion apparatus of the Salmonella Pathogenicity Island (SPI). The ability to invade host epithelial cells requires SPI1, and the ability to survive in host phagocytes requires SPI2 (Groisman and Ochman, 1997). In the present study, we detected the invA gene for the confirmation of Salmonella spp. isolates genetically.

In conclusion, our findings indicated that the prevalence ratio of Salmonella in fish samples was relatively low. Common carp was a reservoir of three types of Salmonella serovars: Enteritidis, Panama, Quinella. Multiple resistances were found in all seven isolates. The Cls1 integrons were detected in all Salmonella isolates as opposed to integrase. As a result, exposure to MDR Salmonella via the common carp food chain is considered a potential risk to human health through foodborne infections with resistant pathogens, and could be horizontally transferable from common carp bacteria to human pathogens due to the presence of integrons/ integrases.

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