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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 persistent circulation of 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 of antimicrobials in human and 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 can interact between different 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 of 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 and 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 tons in Turkey. According to 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 bag incubated at 37°C for 18±2 h. 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 (XLD, CM 0469) 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 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). 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 µM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 1.25 U Taq DNA Polymerase (Fermentas) and 0.5 µM 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 µl volumes containing 5 µl of extracted DNA used as a template; 1X PCR Buffer (Fermentas), 3.25 mM $MgCl_2$ (Fermentas), 200 µM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 2.50 U Taq DNA 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 $MgCl_2$ (Fermentas), 200 µM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 1.25 U Taq 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'- GTG AAA TTA TCG CCA CGT TCG GGC AA -3' R 5'- TCA TCG CAG CGT CAA AGG AAC -3'	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 *et al*. (1999)

Antibiotic susceptibility testing

The isolates were tested against erythromycin (15µg, Oxoid), neomycin (10µg, Oxoid), gentamicin (10µg, Oxoid), tetracycline (10µg, Oxoid), cefotaxime (30µg Oxoid,), amikacin (30µg, Oxoid,), cefalotine (30µg, Oxoid), penicillin (10µg, 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 as 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 impregnated touch sticks (Oxoid, 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 β-lactamase- lactamase 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).

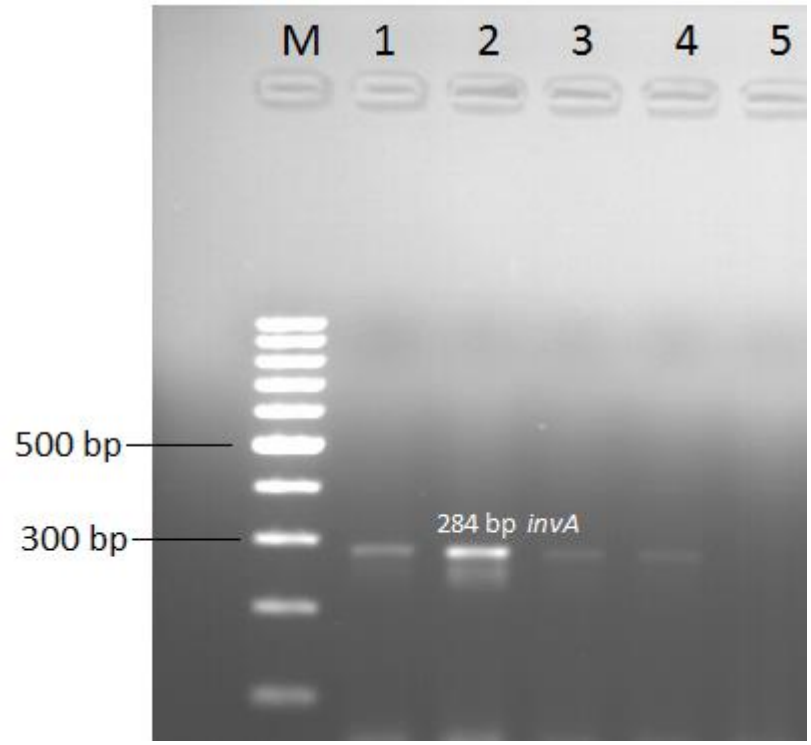

Figure 1: Determination of the *invA* gene in the *Salmonella* strains isolated from Common carp samples using PCR technique. M: Marker; 1, 3 and 4 Lanes; *Salmonella* spp. isolated from Common carp samples, 2 Lane; *S. enteritidis* ATCC 13076 (positive control), 5 Lane; negative isolate.

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 µg) (n=7)	100	R	R	R	R	R	R	R
2	Neomycin (10 µg) (n=3)	42.85	S	R	S	S	S	R	R
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).

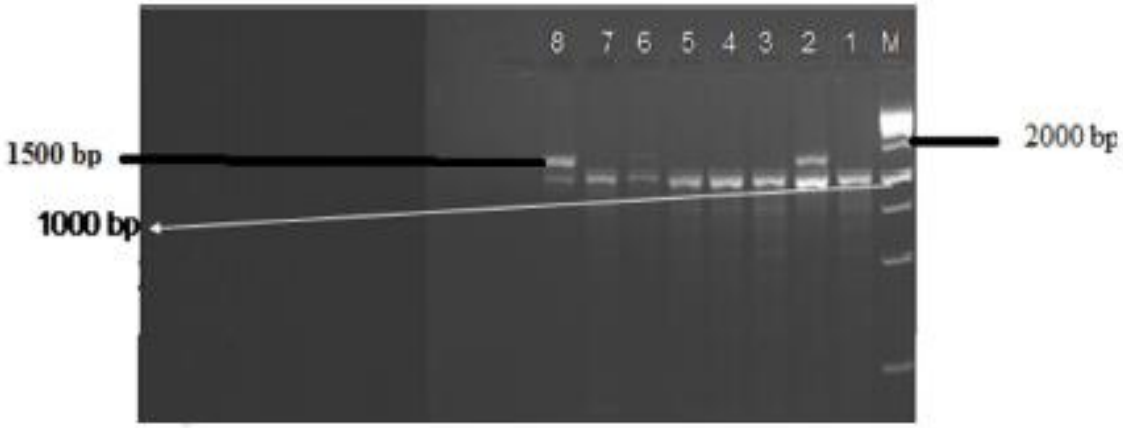

Figure 2: Determination of the Class 1 integron in the *Salmonella* strain sisolated from Common carp samples. Lane M: Marker, Lane 2,6 and 8: 1000 And 1500 bp integrons (two different variable regions-three *S. Panama* isolates); Lane 1: Posıtive control (chicken carcass origin *Salmonella* isolate);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

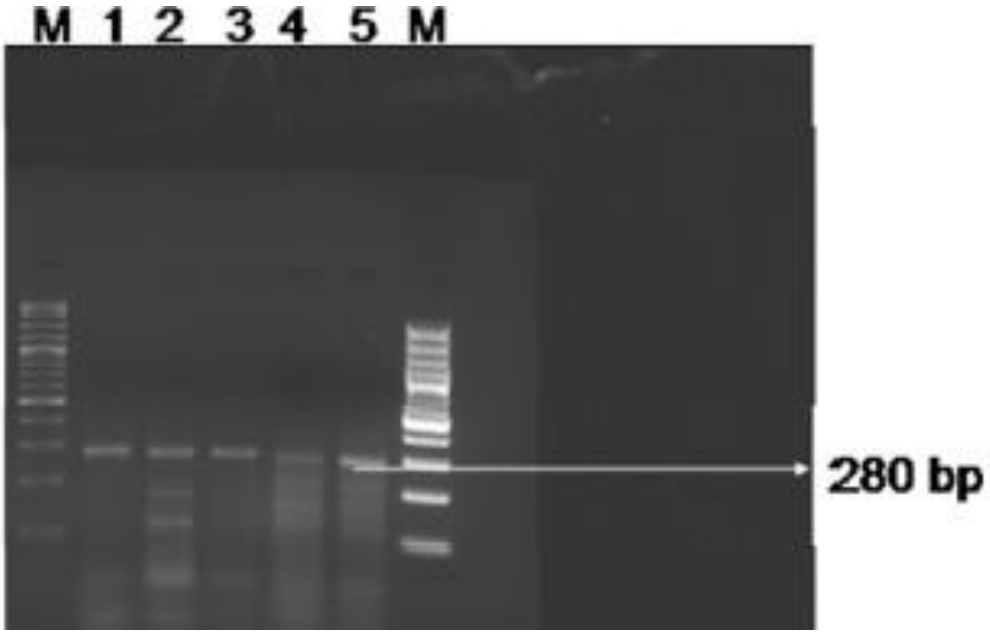

Figure 3: The determination of the integrase (*Intı*) gene in the *Salmonella* strains isolated from Common Carp samples. M: Marker, Lanes 1: Positive control (chicken carcass origin) *Salmonella* isolate), Lane 2, 3, 4 and 5 positive *Salmonella* isolates (*S. enteritis, S. panama* and *S. quinella*, 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 many studies limited to *Salmonella* in fish and contamination results range from 0.0 to 28.2% (BGVV, 1996; 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 of *Salmonella* in freshwater fish, farm-raised 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 source water or farm 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 salted anchovy) samples obtained from the Black Sea, Turkey. In the present study, however, *Salmonella* strains were 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 fishery products was highest in 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 and 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. In addition, *S*. Typhimurium, *S*. Enteritidis, *S*. Newport, and *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). ß-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 ß-lactamase 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 ß-lactamase stable penicillin group (ampicillin and oxacillin and penicillin), one of which was of the lincosamides group (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 to quinolones, 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 are Typhimurium, Heidelberg, Dublin, and Paratyphi B (Andino and Hanning, 2015). 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 of antimicrobial drug resistant serovars that are particularly effective in 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 and cause higher mortality rates compared to susceptible *Salmonella* (Angulo and Mølbak, 2005; Han *et al*., 2011). In terms of multidrug resistance, the most prevalent serovars of epidemiological importance 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 to 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 isolates, two *S. enterica* strains (serovars Bareily and Oslo) that originated from two different countries (Vietnam and India) were resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracycline and chloramphenicol. In another study, it was reported that three *S.* Typhimurium and three *S.* Weltevreden strains were resistant to an array of eight antibiotics which include ampicillin, carbenicillin, doxycycline, kanamicin, nalidixic acid, sulfafurazole, tetracycline and trimethoprim. These results show wide variations. These antibiotics resistant properties of *Salmonella* isolated seafood samples show us that the antibiotics are accumulated in the aquatic environment, which provides the required antibiotic stress for the emergence of more and more antibiotic resistant phenotypes of *Salmonella* due to the indiscriminate use. It has also been 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 seafood link 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.

References

Ahmed, A.M., Nakano, H. and Shimamato, T., 2005. Molecular characterization of integrons in non-typhoid *Salmonella* serovars isolated in Japan: description of an unusual class 2 integron. *Journal of Antimicrobial Chemotherapy*, 55, 371-374.

Andino A. and Hanning I.. 2015. *Salmonella* enterica: Survival, Colonization, and Virulence Differences among Serovars. *The Scientific World Journal*, 2015, 1-16.

Angulo, F., Nargund, V. and Chiller, T., 2004. Evidence of an association between use of antimicrobial agents in food animals and anti-microbial resistance among bacteria isolates from humans and the human health consequences of such resistance. *Journal of Veterinary Medicine B*, 51, 374-379.

Angulo, F.J. and Mølbak, K., 2005. Human health consequences of antimicrobial drug-resistant *Salmonella* and other foodborne pathogens. *Clinical Infectious Diseases*, 41(**11**), 1613-1620.

Antunes, P., Machado, J., Sousa, J.C. and Piexe, L., 2004. Dissemination amongst humans and food products of animal origin of a *Salmonella* Typhimurium clone expressing an integron-borne OXA-30 ß-lactamase. *Journal of Antimicrobial Chemotherapy*, 43, 1-4.

Bakshi, C.S., Singh, V.P., Malik, M., Singh, R.K. and Sharma, B., 2003. 55 kb Plasmid and virulence-associated genes are positively with *Salmonella* Enteritidis pathogenicity in and chickens. *Veterinary Research Communications*, 27, 425-342.

Bass, L., Liebert, C.A., Lee, M.D., Summers, A.O., White, D.G., Thayer, S.G. and Maurer, J.J., 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in Avian *Escherichia coli*. *Antimicrobial Agents Chemotherapy*, 43(**12**), 2925-2929.

BGVV., 1996. Deutscher trendbericht über den verlauf und die quellen von zoonose-infectionen, Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin.

Bhowmick, P.P., Srikumar, S., Devegowda, D., Shekar, M., Ruwandeepika, H.A.D. and Karunasaga, I., 2012. Serotyping and molecular characterization for study of genetic diversity among seafood associated nontyphoidal *Salmonella* serovars. *Journal of Food Protection*, 135(**3**), 371-381.

Buchanan, R.E. and Gibbons, N.E., 1974. Bergey's manual of determinative bacteriology. 8 th ed. Baltimore: The Williams and Wilkins Co., pp. 483-299.

Boyd, D., Cloeckaert, A., Chaslus-Dancla, E. and Mulvey, M.R., 2002. Characterization of variant *Salmonella* genomic island 1 multiple resistance regions from serovars Typhimurium DT104 and Agona. *Antimicrobial Agents Chemotherapy*, 46, 1714- 1722.

Broughton, E.I. and Walker, D.G., 2009. Prevalence of antibiotic-resistant *Salmonella* in fish in Guangdong, China. *Foodborne Pathogens and Disease*, 6(**4**), 519-21.

Center of Disease Control (CDC)., 2013. An Atlas of *Salmonella* in the United States, 1968-2011:

Laboratory-based Enteric Disease Surveillance. Atlanta, Georgia: US Department of Health and Human Services. pp. 1-248.

Chen, G. and Griffiths, M.W., 2001. Detection of *Salmonella* and simultaneous detection of *Salmonella* and Shiga-like toxin-producing *E. coli* using the magnetic capture hybridization polymerase chain reaction. *Letters in Applied Microbiology*, 32, 7-11.

Clinical and Laboratory Standards Institute (CLSI)., 2010. Performance standards for antimicrobial susceptibility testing. Twentieth Informational Supplement, M100-S20.

Di Conza, A.J. and Gutkind, G.O., 2010. Integrons: gene collectors. *Revista Argentina de Microbiología*, 42(**1**), 63-78.

Fangtham, M. and Wilde, H., 2008. Emergence of *Salmonella* paratyphi. A as a major cause of enteric fever: need for early detection, preventive measures, and effective vaccines. *Journal of Travel Medicine*, 15(**5**), 344-350.

Food and Agriculture Organization (FAO)., 2010. Expert workshop on the application of biosecurity Measures to control *Salmonella* Contamınatıon in Sustainable aquaculture FAO Fisheries and Aquaculture Report No. 937 ISSN 2070-6987, Mangalore, India, 19–21 January 2010. pp. 1-46

Food and Agriculture Organization (FAO)., 2011. Better Management Practices for Carp Production in Central and Eastern Europe, The Caucasus and Central Asia. FAO Fisheries and Aquaculture Technical Pape, 566 P.

Firoozeh, F., Zahraei-Salehi, T., Shahcheraghi, F., Karimi, V. and Aslani, M.M., 2012. Characterization of class I integrons among *Salmonella enterica* serovar Enteritidis isolated from humans and poultry. *Immunology and Medical Microbiology*, 64(**2**), 237-243

Flowers, R.S., D'aoust, J-Y, Andrews, W.H. and Bailcy, J.S., 1992. *Salmonella*. In: Compendium of the Methods for the Microbiological Examination of Foods. Ed. 685 P.

Galán, J.E., Ginocchio, C. and Costeas, P., 1992. Molecular and functional characterization of the *Salmonella* Invasion Gene *invA*: Homology of *InvA* to Members of a New Protein Family. *Journal of Bacteriology*, 174(**13**), 4338-4349.

Groisman, E.A. and Ochman, H., 1997. How *Salmonella* became a pathogen. *Trends Microbiology*, 5, 343–349.

Ginocchio, C.C., Rahn, K., Clarke, R.C. and Galan, J.E., **1997.,** Naturally occurring deletions in the centisome 63 pathogenicity island of environmental isolates of *Salmonella* spp. *Infection and Immunity*, 65(**4**)**,** 1267–1272.

Han, J., David, D.E., Deck, J., Lynne, A.M., Kaldhone, P., Nayak, R., Stefanova, R. and Foley, S.L., 2011. Comparison of Salmonella enterica serovar Heidelberg isolates from human patients with those from animal and food sources. *Journal of*

Clinical Microbiology, 49(**3**), 1130-1133.

Hanau-Bercot, B., Podglajen, I., Casin, I. and Collatz, E., 2002. An intrinsic controlelement for translational initiation in class 1 integrons. *Molecular Microbiology*, 44, 119-130.

Hatha, A.A.M. and Lakshmanaperumalsamy, P., 1997. Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. *Food Microbiology*, 14, 111-116.

Heinitz, M.L., Ruble, R.D., Wagner, D.E. and Tatini, S.R., 2000. Incidence of *Salmonella* in fish and seafood. *Journal of Food Protection*, 63, 579-592.

Huss, H.H. and Gram, L., 2004. Characterization of hazards in seafood assurance of seafood. Quality FAO Fisheries Technical Paper. pp. 444 -227.

International Standart Organization (ISO)., 2002. Microbiological of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp. International Standards Organization, ISO (6579). Geneva, Switzerland. pp. 1-50.

Khan, A.A., Cheng, C-M., Van, K.T., West, C.S., Nawaz, M.S. and Khan, S.A., 2006. Characterization of class 1 integron resistance gene cassettes in *Salmonella enterica serovars Oslo* and *Bareily* from imported seafood. *Journal of Antimicrobial Chemotherapy*, 58(**6**), 1308-1310.

Kumar, H.S., Sunil, R., Venugopal, M.N., Kranusagar, I. and Karanusagar, I., 2003. Detection of *Salmonella* spp. in tropical seafood by polymerase chain reaction. *International Journal of Food Microbiology*, 88, 91-95.

Mather, A.E., Reid, S.W.J., Maskell, D.J., Parkhill, J., Fookes, M.C., Harris, S.R., Brown, D.J., Coia, J.E. and Mul, M.R., 2013. Distinguishable epidemics of multidrug-resistant *Salmonella* Typhimurium DT104 in different hosts. *Science*, 341(**6153**), 1514-1517.

Meng, H., Zhang, Z., Chen, M., Su, Y., Li, L., Miyoshi, S.I., Yan, H. and Shi, L., 2011. Characterization and horizontal transfer of class 1 integrons in *Salmonella* strains isolated from food products of animal origin. *International Journal of Food Microbiology*, 149, 274-277.

Miko, A., Pries, K., Schroeter, A. and Helmuth, R., 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from inGermany. *Journal of Antimicrobial Chemotherapy*, 56, 1025-1033.

NARMS (National Antimicrobial Resistance Monitoring System)., 2010. Executive report,2010,http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm312356.htm.

Pao, S., Ettinger, M.R., Khalid, M.F., Reid, A.O. and Nerrie, B.L., 2008. Microbial quality of raw

aquacultured fish fillets procured from internet and local retail markets. *Journal of Food Protection*, 71(**8**), 1544-1549.

Rahn, K., De Grandis, S.A., Clarke, R.C., Mcewen, S.A., Galán, J.E., Ginocchio, C., Curtiss, R. and Gyles, C.L., 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes*, 6(**4**), 271-279.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J. and Woodward, M.J., 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty- five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Antimicrobial Agents and Chemotherapy*, 53, 208-216.

Raufu, I.A., Lawan, F.A., Bello, H.S., Musa, A.S., Ameh, J.A. and Ambali, A.G., 2014. Occurrence and antimicrobial susceptibility profiles of Salmonella serovars from fish in Maiduguri, sub-Saharah, Nigeria. *Egypt Journal of Aquatic Research*, 40, 59-63.

Rodriguez, A., Pangloli, P., Richards, H.A., Mount, J.R. and Draughon, F.A., 2006. Prevalence of *Salmonella* in diverse environmental farm samples. *Journal of Food Protection*, 69(**11**), 2576-2580.

Salehi, T.Z., Mahzounieh, M. and Saeedzadeh, A., 2005. Detection of *InvA* Gene in isolated *Salmonella* from Broilers by PCR Method. *International Journal Poultry Science*, 4(**8**), 557-559.

Shabarinath, S., Kumar, S., Khushiramani, I. and Karunasagar, I., 2007. Detection and characterization of *Salmonella* associated with tropical seafood. *International Journal of Food Microbiology*, 114, 227–233.

Siriken, B., Çadirci, Ö. and İnat, G., 2010. Detection of *Salmonella* spp. in fresh fish, salted anchovies and mussels by the immuno magnetic separation (IMS) and classic culture techniques" The 3rd Global Fisheries and Aquaculture Research Conferance, Egypt, Proceeding for Middle East and North. 29th November-1st December. Foreign Agricultural Relations (FAR), Dokki-Giza, Egypt. *Africa Journal of Animal Science*, 12962(**2**), 159-172.

Sorum, H. and L'abee-Lund, T.M., 2002. Antibiotic resistance in food-related bacteria- a result of interfering with global web of bacterial genetics. *International Journal of Food Microbiology*, 78, 43–56.

Wannaprasata, W., Padungtodb, P. and Chuanchuena, R., 2011. Class 1integrons and virulence genes in *Salmonella* enterica isolates from pork and humans. *International Journal of Antimicrobial Agents*, 37, 457-461.

World Health Organization (WHO)., 2002. Use of antimicrobials outside human medicine and resultant antimicrobial resistance in humans.

Fact sheet N0:268 January 2002. pp. 1-105.

Witte, W., 2002. Selective pressure by antibiotic use in livestock. *International Journal of Antimicrobial Agents*, 16(**Suppl. 1**), 19-24.

Zhang, H., Shi, L., Li, L., Guo, S., Zhang, X., Yamasaki, S., Miyoshi, S. and Shinoda, S., 2004. Identification and characterization of class 1 integron resistance genecassettes among *Salmonella* strains isolated from healthy humans in China. *Microbiology and Immunology*, 48(**9**), 639–645.

Zhao, S., Data, A.R., Ayers, S., Friedman, S., Walker, R.D. and White, D.G., 2003. Antimicrobial-resistant Salmonella serovars isolated from imported foods. *International Journal of Food Microbiology*, 84, 87–92.