Pathogenic microorganisms, heavy metals, and antibiotic residues in seven Korean freshwater aquaculture species

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Abstract This survey was performed to estimate the levels of pathogenic microorganisms, antibiotic residues, and heavy metals in seven Korean freshwater aquaculture species including *Anguilla japonica*, *Cyprinus carpio nudus*, *Oncorhynchus mykiss*, *Pseudobagrus fulvidraco*, *Semisulcospira coreana*, *Silurus asotus*, and *Trionyx sinensis*. None of the ten foodborne pathogens tested in this study were found in any of the species collected from any of the aquaculture farms. Furthermore, no banned chemicals or antibiotic residues were found in any of the species collected from any of the aquaculture farms, except enrofloxacin, which was below guideline limits (0.1 mg/kg). Finally, no species had lead, cadmium, total arsenic, or total mercury concentrations above the Ministry of Food and Drug Safety (MSDF) guidelines (0.5, 0.5, 0.1, and 0.5 mg/kg, respectively). These results ensure the safety of freshwater aquaculture species and will be useful for developing consumption advisories of freshwater fishes.

Keywords: seven Korean freshwater aquaculture species, pathogenic microorganisms, antibiotic residues, heavy metals

Introduction

In Korea, inland freshwater aquaculture is regarded as a very small-scale industry. It comprises an area of 12 km², about 0.02% of the total land area (99,585 km²) and from 2002–2011, freshwater fish aquaculture production accounted for less than 0.75–1% of total fishery production (1). Total inland aquaculture production in 2013 was 28,000 tons, 3.6% lower than in 2012 (1). According to Fisheries Production Statistics (2), eel, trout, and catfish are considered major products, with productions of at least 3,000 t, while soft-shelled turtle and Korean bullhead are minor products with productions of about 250–570 t.

Local festivals and leisure industries provide opportunities to increase the demand for freshwater fish (3) and recent studies examined health benefits (4–6) to improve the competitive edge of freshwater fish for consumption. However, due to sudden increases in freshwater aquaculture imports from China, Vietnam, and other countries, and safety concerns over freshwater aquaculture fish, the inland aquaculture industry in Korea is facing a crisis. In addition, with increasing interest in environmental degradation and protection,

as well as the prevention of freshwater pollution, the requirements for management of inland aquaculture farms have become more stringent.

According to Lee and Yoo (7), the greatest impediment to inland fish consumption is ‘a risk of infection associated with hygiene’, followed by ‘environmental pollution and antibiotic use’, ‘fishy odor’, and ‘anxiety on the country of origin’. Among these concerns, improper antibiotic use, and heavy metals have received attention in freshwater aquaculture.

Antibiotics are used widely to treat and prevent diseases, as well as promote growth and reproduction in fish and other aquaculture species. Widespread and unrestricted antibiotic use in the aquaculture industry to prevent bacterial infection has led to residual amounts in the aquatic environment. This has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments, an increase in antibiotic resistance in fish pathogens, and the transfer of these resistance determinants to human pathogens (8). Moreover, extensive antibiotic use could result in residual antibiotics in fish tissue and products (9). In some cases, even banned chemicals, including malachite green and crystal violet, have been used

unscrupulously. Research on antibiotic residues in cultured fish in Korea has focused mainly on marine fish and only a few studies have examined antibiotic residues in freshwater fish. Jo *et al.* (10) measured fluoroquinolone residue concentrations in carp and eels that were orally administrated fluoroquinolones. In addition, Choi *et al.* (11) surveyed antibiotic residues in freshwater aquaculture fish and monitored 33 prohibited substances and veterinary medicines in eel, rainbow trout, sturgeon, and tilapia. Despite this monitoring, anxieties remain over inland fish consumption. For example, malachite green, a banned substance, was detected in imported eels (12) in 2005 and in freshwater catfish in 2013 (13).

Heavy metals are of concern as they are ubiquitous, non-degradable persistent environmental pollutants and aquatic systems, including rivers, lakes, and inland water reservoirs, are among the systems most affected by metal contamination (14).

In Korea, most research on heavy metals has focused on wild freshwater fish captured from natural habitats, including rivers, lakes, and reservoirs (15-18). Only a few studies have examined the heavy metal content of freshwater aquaculture fish, including research by Kim *et al.* (19) that measured arsenic, cadmium, copper, lead, zinc, and total mercury in 12 wild and farmed freshwater species collected from fish markets, and research by Kim *et al.* (20) that monitored mercury levels in nine freshwater aquaculture fish species.

Greater attention to the safety of freshwater aquaculture animal products is necessary. Based on previous reports, more information is needed on pathogenic microorganisms, antibiotic residues, and heavy metals in Korean freshwater aquaculture species. Therefore, the current study surveyed the safety of seven kinds of Korean freshwater aquaculture species with relatively high commercial importance, including Japanese eel (*Anguilla japonica*), leather carp (*Cyprinus carpio nudus*), rainbow trout (*Oncorhynchus mykiss*), Korean bullhead (*Pseudobagrus fulvidraco*), freshwater snail (*Semisulcospira coreana*), far eastern catfish (*Silurus asotus*), and Chinese softshell turtle (*Trionyx sinensis*) by measuring the levels of pathogenic microorganism and the concentrations of antibiotic residues, and heavy metals.

Materials and Methods

Sampling For six of the commercial aquaculture species, Japanese eel (*Anguilla japonica*), leather carp (*Cyprinus carpio nudus*), rainbow trout (*Oncorhynchus mykiss*), Korean bullhead (*Pseudobagrus fulvidraco*), far eastern catfish (*Silurus asotus*), and softshell turtle (*Trionyx sinensis*), 10 individuals were collected and for freshwater snail (*Semisulcospira coreana*), about 200 individuals were collected from three reservoir locations (Fig. 1). Some parts of these samples were placed in sterilized polyethylene bags (vacuum-packed; 450×650 mm) securely packed in polystyrene containers, and transported at 5°C using frozen ice packs within 4 h for pathogens analysis. The other parts were frozen immediately with the help of fishermen and

Fig. 1. Sampling sites of the seven examined Korean freshwater aquaculture species. *Anguilla japonica* (▲), *Cyprinus carpio nudus* (△), *Oncorhynchus mykiss* (●), *Pseudobagrus fulvidraco* (○), *Semisulcospira coreana* (■), *Silurus asotus* (□), and *Trionyx sinensis* (◆). *Anguilla japonica*: CA (Hwasun, Jeollanam-do), OS (Yeonggwang, Jeollanam-do), and CB (Jeongeup, Jeollabuk-do); *Cyprinus carpio nudus*: GG (Gimje, Jeollabuk-do), BS (Gimje, Jeollabuk-do), and GR (Jeonju, Jeollabuk-do); *Semisulcospira coreana*: SDR (Yesan, Chungcheongnam-do), PC (Yesan, Chungcheongnam-do), and SJG (Gurye, Jeollanam-do); *Silurus asotus*: DH (Gochang, Jeollabuk-do), MD (Buan, Jeollabuk-do), and NS (Iksan, Jeollabuk-do); *Oncorhynchus mykiss*: SM (Jeongseon, Gangwon-do), ERY (Yeongwol, Gangwon-do), and HC (Chungju, Chungcheongbuk-do); *Pseudobagrus fulvidraco*: MC (Gimje, Jeollabuk-do), WD (Gimje, Jeollabuk-do), CMI (Uiwang, Gyeonggi-do); *Trionyx sinensis*: GW (Gunwi, Gyeongsangbuk-do), NJ (Naju, Jeollanam-do), and BN (Gimje, Jeollabuk-do).

were thawed rapidly below 35°C for chemical and heavy metal analysis.

Foodborne pathogens Ten of the foodborne pathogens, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica* in six of the commercial aquaculture species were examined according to the Ministry of Food and Drug Safety (MFDS) (21).

Banned chemicals and antibiotic residue analyses To detect banned chemicals and antibiotic residues, skin and muscle from the harvested freshwater aquaculture organisms were ground and analyzed following the methods of the Food Standard (21) and antibiotic assay for seafood (22). Malachite green, crystal violet,

chloramphenicol, oxytetracycline, chlortetracycline, tetracycline, doxycycline, nalidixic acid, norfloxacin, difloxacin, ciprofloxacin, enrofloxacin, ofloxacin, oxolinic acid, pefloxacin, flumequine, erythromycin, 14 sulfonamides (sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamethoxypyridazine, sulfamerazine, sulfadimidine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole, sulfaquinoxaline, sulfisoxazole, sulfadoxine, sulfaphenazole, and sulfaclozine), cephalixin, amoxicillin, ampicillin, josamycin, kitasamycin, gentamicin, neomycin, trimethoprim, lincomycin, clindamycin, praziquantel, tiamulin, thiamphenicol, and nitrofurantoin metabolites (3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ), semicarbazide (SEM), and 1-aminohydantoin (AHD)) were detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (1290 Infinity II+6460 triple Quadrupole; Agilent Technologies, Santa Clara, CA, USA) with a Poroshell 120 EC-C18 column (3.0×50 mm, 2.7 µm; Agilent Technologies). Colistin was analyzed using LC-MS/MS with a Unison UK-C8 column (2.0×100 mm, 3 µm). Spiramycin, florfenicol, and ormetoprim were analyzed using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (1260 Infinity; Agilent Technologies) and an Eclipse XDB-C18 column (4.6×250 mm, 5 µm; Agilent Technologies). Nitrovin was analyzed using HPLC-UV detection (Agilent 1260) with a Waters Spherisorb® column (5 µm CNRP, 4.6×150 mm; Sigma-Aldrich, St. Louis, MO, USA). Deltamethrin was analyzed using gas chromatography with an electron capture detector (GC-ECD) (Agilent 7890A). Sulfaguanidine was analyzed using HPLC with fluorescence detection (Agilent 1260) and an Eclipse XDB-C18 column (4.6×50 mm, 1.8 µm; Agilent Technologies).

Heavy metal analysis Four heavy metals including lead, total arsenic, cadmium, and total mercury were analyzed following the methods of the Food Standard (21). Ten individuals of similar size and weight from each species were selected and 11 g of muscle were collected. Subsequently, 10 g of the samples were reduced to ash in an oven at 450°C and pulverized. Each ash sample was dissolved in a 5N nitric acid solution until a clear solution (up to 20 mL) was obtained. All samples were measured in triplicate. The concentrations of lead, arsenic, and cadmium were determined by inductively coupled plasma (ICP; Optima 5300 DV; Perkin Elmer, Waltham, MA, USA). Then, 1 g of muscle was used for the determination of mercury using a mercury analyzer (MA-2; Nippon Instruments Corporation, Tokyo, Japan). The detection limits for lead, total arsenic, cadmium, and total mercury were 0.01, 0.1, 0.01, and 0.01 µg/g, respectively.

Statistical analyses The statistical significances of heavy metal concentrations in seven Korean freshwater aquaculture animals were determined by one sample t-test using SPSS (version 12.0, SPSS Inc., Chicago, IL, USA). Statistical significances were set at $p < 0.001$, $p < 0.01$, and $p < 0.05$ against standard values of MFDS.

Results and Discussion

Pathogenic microorganisms in freshwater aquaculture species

Fish is a highly perishable product and spoils due to microbiological activity, chemical oxidation of lipids, and autolysis (23). Moreover, microbial spoilage is the main mechanism that affects fresh fish quality (24,25). In this study, the levels of pathogenic microorganism on seven kinds of Korean freshwater aquaculture species with relatively high commercial importance, including Japanese eel (*Anguilla japonica*), leather carp (*Cyprinus carpio nudus*), rainbow trout (*Oncorhynchus mykiss*), Korean bullhead (*Pseudobagrus fulvidraco*), freshwater snail (*Semisulcospira coreana*), far eastern catfish (*Silurus asotus*), and Chinese softshell turtle (*Trionyx sinensis*) were measured. According to the microorganism standards and specifications of MFDS guideline in Korea, nine of the foodborne pathogens including *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica* must be negative (–) in fish, while *Bacillus cereus* must be below 10^3 CFU/g (21). As results, none of the ten tested foodborne pathogens were found in any of the freshwater aquaculture animals collected from any of the aquaculture farms (Table 1).

The microbial spoilage of cultured freshwater fish is closely related to contamination of fish farms. Contaminated fish ponds (e.g., high bacterial load due to fertilization and human and animal wastewater) enable penetration of pathogens into fish tissue—including gills, the digestive tract, muscles, kidneys, and liver—and may be responsible for food-borne diseases (24,25). Several studies have shown that the concentration of bacteria such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* in water is proportional to the concentration recovered from fish organs and tissue (26,27). According to El-Shafai *et al.* (26), investigation of the microbial quality of tilapia reared in four fecal-contaminated fishponds revealed that all tissue samples except muscle tissues were contaminated with fecal coliforms. The *E. coli* concentration in the digestive tract and muscle of *Jenynsia multidentata* and *Bryconamericus iheringi* was determined by bioassays (27). Field experiments were also conducted with *J. multidentata* collected in the Suquía River, Córdoba, Argentina. The critical bacterial load in water for the recovery of bacteria from muscle was 10^3 CFU/mL for both species. In field experiments, *E. coli* was recovered from the digestive tract and muscle of *J. multidentata* (27).

In this study, none of the ten tested pathogenic bacteria were found in any of the seven freshwater aquaculture species collected from any of the aquaculture farms (Table 1). These results showed that freshwater fish aquaculture farms selected in this study were well controlled from the microbial spoilage. Therefore, it was concluded that foodborne pathogens in these seven freshwater aquaculture species also were able to be well managed.

Table 1. Pathogenic microorganisms in seven Korean freshwater aquaculture animals

	<i>Anguilla japonica</i>	<i>Cyprinus carpio nudus</i>	<i>Onchorhynchus mykiss</i>	<i>Pseudobagrus fulvidraco</i>	<i>Semisulcospira coreana</i>	<i>Silurus asotus</i>	<i>Trionyx sinensis</i>
Total bacterial count (CFU/g)	2.7x10 ³	3.8x10 ²	1.2x10 ²	3.1x10 ³	1.1x10 ³	1.3x10 ³	5.5x10 ¹
<i>Bacillus cereus</i>	¹⁾	-	-	-	-	-	-
<i>Campylobacter jejuni</i>	-	-	-	-	-	-	-
<i>Clostridium perfringens</i>	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-	-
<i>Escherichia coli</i> O157:H7	-	-	-	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-
<i>Salmonella enteritidis</i>	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
<i>Vibrio parahaemolyticus</i>	-	-	-	-	-	-	-
<i>Yersinia enterocolitica</i>	-	-	-	-	-	-	-

¹⁾-, not detected

Banned chemicals and antibiotic residues in freshwater aquaculture species

Most aquaculture fisheries have become high-density, mass production systems to maximize productivity per unit area. As a consequence, aquatic organisms are in constant contact with infectious agents that can cause opportunistic infectious diseases and cannot be maintained antibiotic-free (28). Furthermore, antibiotics prevent infection with bacterial diseases during fish grading, shipment, and distribution.

In addition, the risk of contamination of products by chemical agents is greater in freshwater than in seawater (28,29). Chemical agents such as malachite green and crystal violet are not approved worldwide as veterinary drugs for fish because of their carcinogenicity and mutagenicity (30), but such agents are used illegally due to their low cost and high efficiency (31). Accordingly, consumers are reluctant to consume aquaculture products that might have been exposed to hazardous chemicals or antibiotics. However, few studies have examined the levels of banned chemical and residual antibiotic in freshwater aquaculture fish (11,29). Therefore, we evaluated the levels of banned chemicals and residual antibiotics in seven freshwater aquaculture species.

As results, no banned substances or animal drug residues were found in any of the tested freshwater aquaculture animal samples, except for 0.0305 mg/kg enrofloxacin in *C. carpio nudus*, which was below the standard limit of 0.1 mg/kg (Table 2).

Choi *et al.* (11) investigated drug residues in aquaculture fish, including all 33 banned substances and animal drugs, such as malachite green, tetracycline-based, and fluoroquinolone residues, in eel, rainbow trout, sturgeon, and tilapia before shipment and sale from ten inland aquaculture farms in Gyeonggi-do, Korea in 2009. No banned substances or drug residues were detected in any aquaculture fish except for 0.01, 0.04, and 0.09 mg/kg oxolinic acid in 3 eel samples. According to veterinary medicine guidelines for fish in Korea, the limit for oxolinic acid is 0.1 mg/kg. Since drug residues were undetected or below guideline levels in several cases, the monitored fish samples were considered relatively safe.

Kim *et al.* (29) investigated the levels of residues of malachite

green and antibiotics (tetracycline, oxolinic acid, and ciprofloxacin) in cultured rainbow trout (*Oncorhynchus mykiss*) collected in 22 aquaculture farms (2 in Gyeonggi-do, 12 in Gangwon-do, and 8 in Gyeongsangbuk-do) in January and July 2008 and January and August 2009. Malachite green was not detected in any samples tested in the study. The residual content of tetracycline and ciprofloxacin was less than the permissible amount (<0.2 and <0.1 mg/kg, respectively), while, only one sample exhibited an oxolinic acid content higher than the permissible amount (<0.1 mg/kg). However, oxolinic acid was not detected in any of the tested freshwater aquaculture animal samples, including eel and rainbow trout, in our study.

Among the aspects of fish and fishery product quality, more than 80% of Japanese consumers seem to be aware of residual antibiotics, food additives, and contaminants (32). Abe and Fuchino (33) performed a survey of residual antibiotic agents—including oxytetracycline, thiamphenicol, sulfamerazine, sulfadimidine, sulfamonomethoxine, sulfadimidine, sulfaquinoxaline, oxolinic acid, and ormetoprim—in cultured fish and shellfish (19 species) purchased from the Tokyo Central Market from April 1998 to May 1999. Of 240 samples tested, oxytetracycline (OTC) was detected in 14, which comprised flounder (*Paralichthys olivaceus*), yellowtail (*Seriola quinqueradiata*, hamachi), seriola (*Seriola purpurascens*, kanpachi) and freshwater eel (*Anguilla japonica*, unagi). OTC was detected in livers of all yellowtail, seriola, and freshwater eels at concentrations higher than that in muscle or skin. Otherwise, no residual antibiotic was detected in 24 samples of freshwater fishes, *Plecoglossus altivelis* (sweet smelt), *Salmo gairdneri* (rainbow trout), and *Salvelinus* spp. (trout) or in the freshwater reptile *Trionyx sinensis* (softshell turtle).

Done and Halden (34) investigated the presence of 47 antibiotics in shrimp, salmon, catfish, trout, tilapia, and swai purchased in the United States originating from 11 different countries. The samples were collected from retail grocery stores in Arizona and California (southwest US) over a period of 3 months from June to August 2012. Five antibiotics were present at levels above the limits of detection of the US FDA regulations: OTC (in wild shrimp, 7.7 mg/kg fresh weight;

Table 2. Concentration of banned chemicals and antibiotic residues (mg/kg fresh weight) for seven Korean freshwater aquaculture animals

Chemical and antibiotics	Standard (mg/kg)	<i>Anguilla japonica</i>	<i>Cyprinus carpio nudus</i>	<i>Onchorhynchus mykiss</i>	<i>Pseudobagrus fulvidraco</i>	<i>Silurus asotus</i>	<i>Semisulcospira coreana</i>	<i>Trionyx sinensis</i>
Malachite Green	ND ¹⁾	ND	ND	ND	ND	ND	ND	ND
Crystal Violet	ND	ND	ND	ND	ND	ND	ND	ND
Chloramphenicol	ND	ND	ND	ND	ND	ND	ND	ND
Total amount of OTC/CTC/TC*	0.2	ND	ND	ND	ND	ND	ND	ND
Doxycycline	0.05	ND	ND	ND	ND	ND	ND	ND
Spiramycin	0.2	ND	ND	ND	ND	ND	ND	ND
Total amount of enrofloxacin/ciprofloxacin	0.1	ND (<LOQ) ²⁾	0.0305 (enrofloxacin)	ND (<LOQ)	ND	ND (<LOQ)	ND (<LOQ)	ND
Erythromycin	0.2	ND	ND	ND	ND	ND	ND	ND
Deltamethrin	0.03	ND	ND	ND	ND	ND	ND	ND
Florfenicol	0.2	ND	ND	ND	ND	ND	ND	ND
Total amount of 15 sulfonamides	0.1	ND	ND	ND	ND	ND	ND	ND
Cephalexin	0.2	ND	ND	ND	ND	ND	ND	ND
Amoxicillin	0.05	ND	ND	ND	ND	ND	ND	ND
Ampicillin	0.05	ND	ND	ND	ND	ND	ND	ND
Oxolinic acid	0.1 ^{a,d}	ND	ND	ND (<LOQ)	ND	ND	ND	ND (<LOQ)
Flumequine	0.5	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)
Nalidixic acid	0.03	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)
Difloxacin	0.3	ND	ND	ND(< LOQ)	ND	ND	ND	ND
Josamycin	0.05	ND	ND	ND	ND	ND	ND	ND
Kitasamycin	0.2	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	ND
Ofloxacin	ND	ND (<LOQ)	ND (<LOQ)	ND (<LOQ)	ND	ND	ND (<LOQ)	ND (<LOQ)
Pefloxacin	ND	ND (<LOQ)	ND	ND (<LOQ)	ND	ND	ND	ND (<LOQ)
Gentamicin	0.1 ^{a,d}	ND	ND	ND	ND	ND	ND	ND
Neomycin	0.5	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	0.05	ND (0.0002) (<LOQ)	ND (0.0000) (<LOQ)	ND	ND	ND	ND (0.0002) (<LOQ)	ND (0.0000) (<LOQ)
Clindamycin	0.1 ^c	ND	ND	ND	ND	ND	ND	ND
Lincomycin	0.1	ND	ND	ND	ND	ND	ND	ND
Tiamulin	0.1	ND	ND	ND	ND	ND	ND	ND
Praziquantel	0.02	ND	ND	ND	ND	ND	ND	ND
Thiamphenicol	0.05 ^{a,b}	ND	ND	ND	ND	ND	ND	ND
Ormetoprim	0.1	ND	ND	ND	ND	ND	ND	ND
Colistin	0.15	ND	ND	ND	ND	ND	ND	ND
Total amount of AOZ/AMOX/ADH/SEM/Nitrovin**	ND	ND	ND	ND	ND	ND	ND	ND

¹⁾ND, Not detected²⁾<LOQ, Less than limit of quantitationa, additional analysis item for *Onchorhynchus mykiss*b, additional analysis item for *Silurus asotus*c, additional analysis item for *Anguilla japonica*d, additional analysis item for *Cyprinus carpio nudus*

*OTC, chlortetracycline; CTC, oxytetracycline; TC, tetracycline

**AOZ, 3-amino-2-oxazolidinone; AMOX, 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one; SEM, semicarbazide; AHD, 1-aminohydantoin

farmed tilapia, 2.7; farmed salmon, 8.6; farmed trout with spinal deformities, 3.9), 4-epioxytetracycline (farmed salmon, 4.1), sulfadimethoxine (farmed shrimp, 0.3), ormetoprim (farmed salmon, 0.5), and virginiamycin (farmed salmon marketed as antibiotic-free, 5.2). OTC was the most commonly detected antibiotic compound. However, OTC was not detected in any of the freshwater aquaculture animal samples tested in this study.

Heavy metal concentrations in freshwater aquaculture species

Heavy metals are dangerous due to their toxicity, bioaccumulation, and persistence, and heavy metal pollution has become a global issue in recent decades (35). Freshwater aquatic systems, including lakes, streams, and rivers, have become polluted by heavy metals (36). In addition, some heavy metals, including arsenic, mercury, cadmium, and lead, are severe toxins. Freshwater aquaculture species are exposed to heavy metals via four major routes: food consumption, water uptake by gills, non-edible particle consumption, and dermal absorption. Once absorbed, heavy metals enter the bloodstream and are distributed to various organs, including the liver, kidneys, and gills, before elimination or storage (37).

The lead, total arsenic, cadmium, and total mercury concentrations in the seven freshwater aquaculture species are presented in Table 3. The lead concentrations of *A. japonica*, *C. carpio nudus*, *O. mykiss*, *P. fulvidraco*, *S. coreana*, *S. asotus*, and *T. sinensis* were 0.039 (0.013–0.090), 0.010 (0.006–0.014), 0.019 (0.000–0.060), 0.016 (0.000–0.060), 0.156 (0.080–0.259), 0.021 (0.010–0.047), and 0.007 (0.002–0.010) mg/kg, respectively. The cadmium concentrations of *A. japonica*, *C. carpio nudus*, *O. mykiss*, *P. fulvidraco*, *S. asotus*, *S. coreana*, and *T. sinensis* were 0.022 (0.006–0.050), 0.013 (0.004–0.020), 0.010 (0.000–0.030), 0.010 (0.006–0.020), 0.099 (0.060–0.140), 0.010 (0.006–0.020), and 0.006 (0.000–0.011) mg/kg, respectively. The total arsenic concentrations of *A. japonica*, *C. carpio nudus*, *O. mykiss*, *P. fulvidraco*, *S. asotus*, *S. coreana*, and *T. sinensis* were 0.023 (0.000–0.100), 0.013 (0.000–0.050), 0.023 (0.000–0.090), 0.029 (0.000–0.140), 0.031 (0.000–0.180), 0.023 (0.000–0.090), and 0.016 (0.000–0.060) mg/kg, respectively. The total mercury concentrations of *A. japonica*, *C. carpio nudus*, *O. mykiss*, *P. fulvidraco*, *S. asotus*, *S. coreana*, and *T. sinensis* were 0.309 (0.180–0.387), 0.246 (0.120–0.443), 0.193 (0.040–0.299), 0.295 (0.110–0.362), 0.233 (0.050–0.388), 0.452 (0.070–0.477), and 0.226 (0.140–0.315) mg/kg, respectively. In all seven species, the heavy metal concentrations were significantly below the concentrations set by the MFDS guidelines (21).

Previous studies measured lead, cadmium, arsenic, and mercury concentrations in freshwater fish captured from rivers or lakes, or collected from fish markets. Kim *et al.* (19) found an average lead concentration in freshwater fish of 0.026 (range ND–0.423) mg/kg and no lead was detected in masou salmon (*Oncorhynchus masou*) and sweetfish (*Plecoglossus altivelis*), while the highest lead concentration was measured in mud loach (*Misgurnus mizolepis*) at 0.172 mg/kg. Kim and Han (38) found an average lead concentration

of 0.075 mg/kg in 11 freshwater fishes. In research by Sheo *et al.* (39), the lead concentration in freshwater fish, including Crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), *S. asotus*, and snakehead (*Channa argus*), ranged from 0.666–1.984 mg/kg. In this study, the average lead concentration was 0.0383 mg/kg, the highest average lead concentration was found in *S. coreana*, at 0.156 mg/kg, and the lowest was in *P. sinensis*, at 0.007 mg/kg. The lead concentrations in this study were similar to those of previous studies that examined freshwater fish from rivers. The lead concentrations were lower than the limit of 0.5 mg/kg set by the MFDS guidelines (21).

Regarding cadmium, Kim *et al.* (19) found an average concentration in the surveyed freshwater fish of 0.011 (ND–0.086) mg/kg, with eels having the highest average cadmium concentration of 0.028 mg/kg and trout the lowest at 0.001 mg/kg. Kim and Han (38) found an average cadmium concentration in freshwater fish of 0.002 (ND–0.050) mg/kg. Kim *et al.* (17) measured cadmium concentrations of 0.1256–0.1935 mg/kg in *C. auratus*. Sheo *et al.* (39) found that the cadmium concentration in *C. auratus*, *C. carpio*, *S. asotus*, and *C. argus* ranged from 0.017–0.052 mg/kg. In the species surveyed in this study, the average cadmium concentration was 0.0243 mg/kg; the highest average concentration was found in *S. coreana* at 0.099 mg/kg and the lowest in *T. sinensis* at 0.006 mg/kg. The range in cadmium concentrations in this study was comparable to those of previous studies. The cadmium concentrations were lower than the limit of 0.5 mg/kg set by the MFDS guidelines (21).

Regarding arsenic, Kim *et al.* (19) found an average concentration in freshwater fish of 0.370 (0.024–2.231) mg/kg; sea rundace (*Tribolodon hakonensis*) had the highest average arsenic content (1.362 mg/kg), while *C. carpio nudus* had the lowest (0.060 mg/kg). In this study, the average total arsenic in the seven species was 0.0226 mg/kg; the highest average arsenic concentration was found in *S. coreana* at 0.031 mg/kg and the lowest was in *C. carpio nudus* at 0.013 mg/kg. The range of arsenic detected in this study was similar to those of previous studies. The arsenic concentrations were lower than the limit of 0.1 mg/kg set by the MFDS guidelines (21).

Finally, Kim *et al.* (20) measured mercury concentrations in *A. japonica*, *C. argus*, *M. mizolepis*, *C. carpio nudus*, *C. carpio*, *O. masou*, *S. asotus*, *P. altivelis*, and *C. auratus* of 0.084, 0.077, 0.060, 0.056, 0.046, 0.035, 0.028, 0.021, and 0.012 mg/kg wet weight, respectively. These concentrations were below 0.50 mg/kg, the limit set by the Korean Food and Drug Administration (21). Kim *et al.* (19) found an average mercury concentration in surveyed freshwater fish of 0.058 (0.006–0.349) mg/kg; the highest average concentration was found in snakehead (*C. argus*) at 0.103 mg/kg, while the lowest was in *C. carpio nudus* at 0.009 mg/kg. The average mercury concentration of 11 freshwater fishes collected from fish markets was 0.053 (ND–0.237) mg/kg (33). Mercury concentrations of 0.0167–0.0407 mg/kg (17) and 0.07 (0.02–0.15) mg/kg (40) have been measured in *C. auratus*. Sheo *et al.* (39) found mercury concentrations of 0.020–0.139 mg/kg in *C. auratus*, *C. carpio*, *S. asotus*, and *C. argus*. In this

Table 3. Heavy metal concentrations (mg/kg fresh weight) in seven Korean freshwater aquaculture animals

Heavy metal	Standard (mg/kg)	<i>Anguilla japonica</i>	<i>Cyprinus carpio nudus</i>	<i>Onchorhynchus mykiss</i>	<i>Pseudobagrus fulvidraco</i>	<i>Semisulcospira coreana</i>	<i>Silurus asotus</i>	<i>Trionyx sinensis</i>
Lead	0.5	0.039±0.035 ^{1a} (0.013-0.090)	0.010±0.003 ^a (0.006-0.014)	0.019±0.028 ^a (0.000-0.060)	0.016±0.030 ^a (0.000-0.060)	0.156±0.079 ^a (0.080-0.259)	0.021±0.017 ^a (0.010-0.047)	0.007±0.004 ^a (0.002- 0.010)
Cadmium	0.5	0.022±0.019 ^a (0.006-0.050)	0.013±0.008 ^a (0.004-0.020)	0.010±0.014 ^a (0.000-0.030)	0.010±0.007 ^a (0.006-0.020)	0.099±0.034 ^a (0.060-0.140)	0.010±0.007 ^a (0.006 -0.020)	0.006±0.005 ^a (0.000-0.011)
Total arsenic	0.1	0.023±0.043 ^c (0.000-0.100)	0.013±0.025 ^b (0.000-0.050)	0.023±0.045 ^c (0.000-0.090)	0.029±0.055 ^c (0.000-0.140)	0.031±0.066 ^c (0.000-0.180)	0.023±0.045 ^c (0.000-0.090)	0.016±0.029 ^c (0.000-0.060)
Total mercury	0.5	0.309±0.113 ^c (0.180-0.387)	0.246±0.144 ^c (0.120-0.443)	0.193±0.110 ^c (0.040-0.299)	0.295±0.235 ^c (0.110-0.362)	0.233±0.140 ^c (0.050-0.388)	0.452±0.164 ^c (0.070-0.477)	0.226±0.076 ^b (0.140-0.315)

¹Statistical significances were expressed as ^a, ^b, and ^c at the levels of $p < 0.001$, $p < 0.01$, and $p < 0.05$ against the standard values set by the MFDS guidelines, respectively.

study, the average mercury concentration was 0.335 mg/kg; *Silurus asotus* had the highest average mercury concentration (0.452 mg/kg) and *O. mykiss* had the lowest (0.193 mg/kg). The concentration range of mercury in this study was similar to those of previous studies. Furthermore, the mercury concentrations were lower than the limit of 0.5 mg/kg set by the MFDS guidelines (21). Many studies have shown that fish mercury concentrations depend on the concentration in their food (41,42). Therefore, it is necessary to monitor and manage the mercury content in aquaculture feed to maintain safe levels.

In this study, the concentrations of the heavy metals in muscle tissue were compared for each species. In *A. japonica*, the metal concentration was Hg>Pb>AS>Cd. In *C. carpio nudus*, Hg>AS=Cd>Pb. In *O. mykiss*, *P. fulvidraco*, *S. asotus*, and *P. sinensis*, metal concentrations were Hg>AS>Pb>Cd. Finally, in *S. coreana*, the metal concentration was Hg>Pb>Cd>AS (Table 3). In all seven species, mercury was present at the highest concentration of the heavy metals examined, while there were no other consistent trends among the other heavy metals. All heavy metal concentrations were lower than the MFDS guidelines for heavy metals in fish for human consumption (21).

There is concern over the safety of freshwater aquaculture products, and these organisms require more extensive and systematic monitoring and sustainable management to remove food pathogens, heavy metals, and medicine residues from the production procedure. To promote the freshwater aquaculture industry, several studies investigated functional materials in seven Korean freshwater aquaculture species, including *A. japonica*, *C. carpio nudus*, *O. mykiss*, *P. fulvidraco*, *S. coreana*, *S. asotus*, and *T. sinensis* (4–6). However, it was still necessary to confirm the safety of these species. Therefore, in this study, the concentrations of pathogenic microorganisms, heavy metals, and antibiotic residues in these freshwater aquaculture species were determined. In these results, foodborne pathogens, heavy metals, banned chemicals, and antibiotic residues were not found in seven species of freshwater aquaculture animals collected from a number of aquaculture farms in Korea. Despite high consumption of *S. coreana* and *T. sinensis* and much biological research on various freshwater aquaculture fish species, no study

had previously examined heavy metals and antibiotic residues in *S. coreana* and *T. sinensis*. To our knowledge, this is the first report on the monitoring of heavy metal and antibiotic residue in these species in Korea.

This study has several limitations. It is a small-scale survey study with a low number of aquaculture farms, animal sample-sizes, and heavy metals tested. Larger scale studies with longer survey periods, larger sample-sizes, more heavy metals, and more extensive aquaculture farm surveys are needed to confirm the results. In conclusion, the safety of freshwater aquaculture species was verified regarding levels of foodborne pathogens, heavy metals, banned substances, and drug residues. The results of this study will be useful for developing consumption advisories for these species.

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