Hindawi
Aquaculture Research
Volume 2023, Article ID 5014754, 9 pages
https://doi.org/10.1155/2023/5014754

Research Article

Antimicrobial Resistance of Fecal-Indicator Bacteria Isolated from Aquatic Animal Farms along the Korean Coast

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Received 8 November 2022; Revised 5 January 2023; Accepted 17 February 2023; Published 6 March 2023

Academic Editor: Mohamed Abdelsalam

The safety of seafood is a critical public health concern in Korea because of the high rate of raw seafood consumption. We investigated the prevalence and antimicrobial resistance of fecal-associated bacteria (*Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus aureus*) in 50 seawater samples and 48 aquatic animals collected from aquaculture farms along the Korean coast in 2018. Of them, *E. coli* was the most prevalent in seawater (24.0%) and aquatic animals (18.8%). Although more than 80.0% of *E. coli* isolates were sensitive to 9 of 15 antimicrobials, approximately 20.0% of the isolates were resistant to 4 antimicrobials, including tetracycline and streptomycin. *Enterococcus* spp. isolates (2.7–32.0%) were resistant to only 5 of 12 antimicrobials. Notably, 30.1% of *E. coli* isolates were resistant to three or more antimicrobials. To minimize health risks associated with raw seafood consumption, more research is needed concerning the prevalence and antimicrobial resistance of fecal indicators.

1. Introduction

Escherichia coli and *Enterococcus* spp. are often found in food supplies. They are commonly used as fecal indicators because of their ubiquitous nature in human and animal feces [1–4]. Fecal indicators are used to determine the hygienic quality of water and food products [5–7]. Fecal-associated microorganisms (e.g., fecal-indicator bacteria, *Salmonella* spp., and *Staphylococcus aureus*) may be transported through land-based fecal pollution sources [8–10]; they can contaminate and negatively affect the sanitary status of aquatic products farmed in coastal regions.

In particular, antimicrobial resistance has become a global public health priority [11, 12]. This problem is attributed to the widespread and inappropriate use of antibiotics to prevent and treat bacterial infections in clinical settings, as well as in agricultural and aquaculture systems [13–15]. Furthermore, the level of antibiotic resistance in *E. coli* is regarded as a good indicator of the selection pressure exerted by the use of antibiotic agents [6].

Fishery products are a major food resource worldwide with important roles in human nutrition. According to the Food and Agriculture Organization of the United Nations [16], world fishery production continues to grow because of increased aquaculture production. Additionally, Statistics Korea [17] reported that aquaculture production, including seaweed, has increased by approximately 330% over the past 20 years, reaching over 2.3 million tons in 2020. Korea is one of the leading countries in the consumption of fishery products. Large amounts of fishery products, particularly raw products, are consumed in Korea [18]. In 2019, the estimated per capita consumption of seafood in Korea was 69.9 kg per year, including aquatic animals (42.3 kg per year) and seaweeds (27.6 kg per year) [19]. Therefore, the safety and quality of fishery products is a critical public health concern in Korea.

Aquatic animals are extensively cultured along the southern and western coasts of Korea, particularly in the region selected for this study [18]. The monitoring of antimicrobial resistance in human and animal fecal-associated

microorganisms is necessary for the implementation of proper public health measures. Such a study is important because of the widespread consumption of raw uncooked seafood in Korean culture. In this study, we investigated the distribution and antimicrobial susceptibility of fecal-associated microorganisms in aquatic animals (finfish and shrimp) and culture water from major aquaculture farms along the Korean coast.

2. Materials and Methods

2.1. Sample Collection. The aquaculture farms selected for this study are in the major aquaculture production areas of Korea [18]. Samples of water and aquatic animals (fish and shrimp) were collected from eight commercial aquaculture farms along the Korean coast in 2018 (Figure 1). Fish samples were purchased from April to November from six fish farms, including Korean rockfish (*Sebastes schlegeli*; stations 1 and 3–6) and red seabream (*Pagrus major*; station 2). Samples of whiteleg shrimp (*Litopenaeus vannamei*) were purchased from May to November from two farms (stations 7 and 8). At the time of aquatic animal sampling, water samples were also collected from eight farms. In total, 98 samples were collected from eight fixed stations, including 48 aquatic animal samples (8 red seabreams, 29 Korean rockfish, and 11 whiteleg shrimp) and 50 seawater samples (Table 1). Some samples could not be collected because of bad weather (e.g., rainfall and high temperature) or complete harvest of aquatic animals. All samples were kept in a cooler during transport to the laboratory. The water temperature was measured at the seawater sampling stations (1–4) from April to November, 2018 [18].

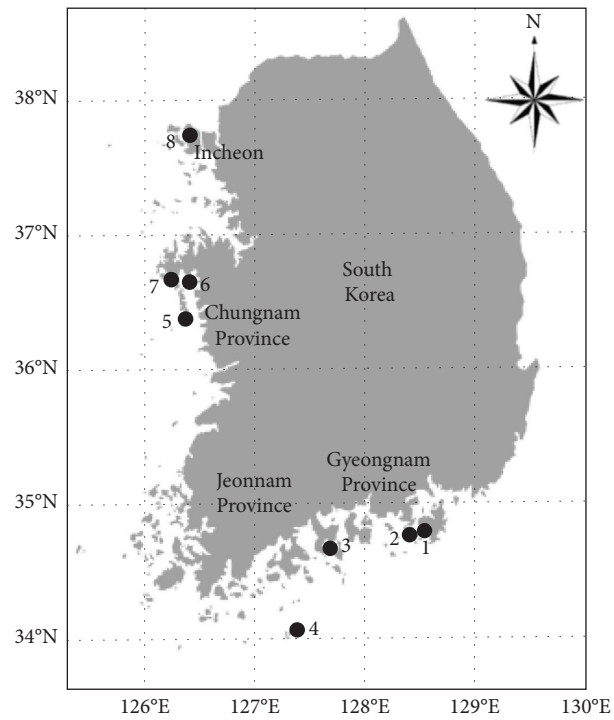

Figure 1: Locations of commercial aquaculture farms reported in this study.

2.2. Analysis of Fecal-Associated Microorganisms. All samples used for the isolation of fecal-associated bacteria (e.g., *E. coli*, *Enterococcus faecium*, *Enterococcus faecalis*, and *S. aureus*) were immediately analyzed upon arrival at the laboratory. For bacterial isolation, both the intestine and gill from each collected fish were separated and homogenized using a blender (Waring, Torrington, CT, USA) [18]. Tissue samples of shrimp were homogenized after shell removal.

E. coli strains were isolated in accordance with the modified ISO/TS 16649-3 method [20]. Briefly, 25 g of the animal tissue homogenate or 25 mL of seawater sample was placed in 225 mL of EC medium broth (Difco, Detroit, MI, USA), and then incubated for 18–24 h at 37°C for enrichment. To isolate *E. coli* strains, approximately, 10μL aliquots of each positive culture were streaked onto five plates of tryptone bile X-glucuronide agar (Merck, Darmstadt, Germany), and then incubated for 18–24 h at 44°C. Subsequently, 3–5 blue or blue-green colonies suspected to be *E. coli* were picked from each tryptone bile X-glucuronide agar plate.

Enterococcus species (*E. faecium* and *E. faecalis*) were isolated in accordance with the method established by Sung et al. [1]. Briefly, 25 g of the animal tissue homogenate or 25 mL of seawater sample was placed in 225 mL of Azide dextrose broth (Merck) containing 6.5% NaCl, and then incubated for 48 h at 37°C for enrichment. Next, approximately 10μL aliquots of each positive culture were streaked onto five Enterococcosel agar plates (Difco), and then incubated for 48 h at 37°C. Subsequently, 3–5 black colonies suspected to be *Enterococcus* spp. were picked from each Enterococcosel agar plate.

Finally, the method described in the Korea Food Code [21] was used to isolate *S. aureus* strains. Briefly, 25 g of the animal tissue homogenate or 25 mL of seawater sample was placed in 225 mL of tryptic soy broth (Merck, Darmstadt, Germany) containing 10.0% NaCl, and then incubated for 18–24 h at 37°C for enrichment. Next, approximately 10-μL aliquots of each positive culture were streaked onto five plates of Baird Parker agar (Merck) containing egg yolk, and then incubated for 18–24 h at 37°C. Subsequently, 3–5 black colonies suspected to be *S. aureus* were picked from each Baird Parker agar plate.

Thereafter, all presumptive strains of *E. coli*, *Enterococcus* spp., and *S. aureus* were confirmed using the VITEK system (BioMerieux Vitek, Marcy l'Etoile, France). All confirmed isolates (*E. coli* strains, $n = 302$; *Enterococcus* spp. strains, $n = 81$) are listed in Supplementary Tables 1–4. The isolates were inoculated onto tryptic soy agar slants, incubated for 18–24 h at 37°C, and stored at 0–4°C for further testing.

2.3. Antimicrobial Susceptibility Testing of Fecal-Indicator Bacteria. In accordance with the guidelines of the Clinical and Laboratory Standards Institute [22] and the U.S. Food and Drug Administration [23], the antimicrobial susceptibility of the fecal-indicator bacteria isolates was determined. Of the original confirmed isolates stored at 0–4°C, only

Table 1: Distributions of fecal-indicator bacteria in water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018.

Type	Total number	Samples			
		Positive number (%)			
		Escherichia coli	*Enterococcus* species		
			E. faecium	*E. faecalis*	Subtotal
Water	50	12 (24.0)	2 (4.0)	1 (2.0)	3 (6.0)
Fish farms	38	11 (28.9)	2 (5.3)	1 (2.6)	3 (7.9)
Shrimp farms	12	1 (8.3)	0 (0)	0 (0)	0 (0)
Aquatic animals	48	9 (18.8)	3 (6.2)	2 (4.2)	5 (10.4)
Fish	37	8 (21.6)	2 (5.4)	1 (2.7)	3 (8.1)
Shrimp	11	1 (9.1)	1 (9.1)	1 (9.1)	2 (18.2)
Total	98	21 (21.4)	5 (5.1)	3 (3.1)	8 (8.2)

Staphylococcus aureus was not detected in any of the samples.

E. coli ($n = 289$) and *Enterococcus* spp. ($n = 75$) isolates could be retrieved and were used for further testing. Antimicrobial susceptibility testing of fecal-indicator bacteria was performed as microbroth dilution MIC with the Sensititre® microbroth dilution system (Trek Diagnostic Systems Ltd., East Grinstead, UK), according to the manufacturer's instructions and the method of Mok et al. [18].

The following 15 antimicrobials were used for *E. coli*, with a range of concentrations (μg/mL) shown in parentheses: gentamicin (GEN; 1–64), streptomycin (STR; 16–128), amoxicillin/clavulanic acid (AMC; 2–32 and 1–16), meropenem (MEM; 0.25–4), cefepime (FEP; 0.25–16), cefoxitin (FOX; 1–32), ceftazidime (CAZ; 1–16), trimethoprim/sulfamethoxazole (SXT; 0.12–4 and 2.38–76), sulfisoxazole (FIS; 16–256), ampicillin (AMP; 2–64), chloramphenicol (CHL; 2–64), colistin (CL; 2–16), ciprofloxacin (CIP; 0.12–16), nalidixic acid (NA; 2–128), and tetracycline (TET; 2–128). In addition, the following 12 antimicrobials were used for *Enterococcus* species: GEN (128–2048), STR (128–2048), vancomycin (VAN; 2–32), tigecycline (TGC; 0.12–4), daptomycin (DAP; 1–32), erythromycin (ERY; 1–64), linezolid (LNZ; 1–16), AMP (1–64), CHL (2–32), CIP (0.25–16), quinupristin/dalfopristin (SYN; 1–32), and TET (2–128).

The results were classified as resistant (R), intermediately resistant (I), or susceptible (S) based on the MIC interpretive criteria suggested by the CLSI [22]. Interpretive criteria not available from the CLSI were derived from the breakpoint (STR) for *E. coli*, and the breakpoints (GEN, STR, and TGC) for *Enterococcus* species suggested by the US FDA [23]. *E. coli* ATCC 25922 was used as a quality control strain. The multiantimicrobial resistance (MAR) index of the isolates was defined as x/y, where x represents the number of antimicrobial agents to which the isolate was resistant and y represents the total number of antimicrobial agents against which an individual isolate was tested [24].

2.4. Statistical Analysis. All statistical analyses were performed using R software for Windows [25]. Duncan's multiple-range tests were used to compare differences between bacterial occurrences and/or antimicrobial resistance patterns at a 95% confidence level with the "agricolae package" in the R program.

3. Results and Discussion

3.1. Distributions of Fecal-Associated Microorganisms in Water and Aquatic Animals. Table 1 and Supplementary Tables 1–4 show the distributions of fecal-indicator microorganisms (e.g., *E. coli*, *E. faecium*, *E. faecalis*, and *S. aureus*) isolated from water samples and aquatic animals (fish and shrimp) obtained from commercial aquaculture farms along the Korean coast from April to November, 2018. Of 50 water samples from 8 stations, the fecal-indicator strains *E. coli*, *E. faecium*, and *E. faecalis* were detected in 12 (24.0%), 2 (4.0%), and 1 (2.0%) samples, respectively. Of 48 aquatic animal samples, *E. coli*, *E. faecium*, and *E. faecalis* were detected in 9 (18.8%), 3 (6.2%), and 2 (4.2%) samples, respectively. No *S. aureus* isolates were found in any samples of water or aquatic animals. Among the fecal-indicator bacteria tested in this study, *E. coli* was the most abundant species. In a similar analysis, *E. coli* was the most prevalent fecal-indicator bacteria in both sardines and shrimp (32% and 66%, respectively) from different fishmongers in Algeria [10]. Our study demonstrated that the levels of contamination with *Enterococcus* spp. were low. Only 10.4% of aquatic animals (fish and shrimp) were contaminated (Table 1), similar to the findings in a study that reported a low detection rate (18.8%) in retail sashimi (raw fish) in Korea [1].

The monthly variations of *E. coli* isolated from water samples and aquatic animals from commercial aquaculture farms along the Korean coast in 2018 are shown in Figure 2 and Supplementary Table 1. The monthly detection rates of *E. coli* strains in water samples ranged from 0% to 60% from April to November, with rates of more than 50% in June and September (Figure 2(a) and Supplementary Table 1). In the aquatic animal samples, the monthly detection rates of *E. coli* strains ranged from 0% to 33.3%, with the maximum levels found in May and November (Figure 2(b) and Supplementary Table 1). Although the monthly detection rates of *E. coli* tended to differ between water samples and aquatic animals, the overall differences were not statistically significant.

Of note, the highest rate of detection of *E. coli* strains in water samples was in September (60.0%), followed by June (57.1%) and August (40.0%); conversely, *E. coli* was not

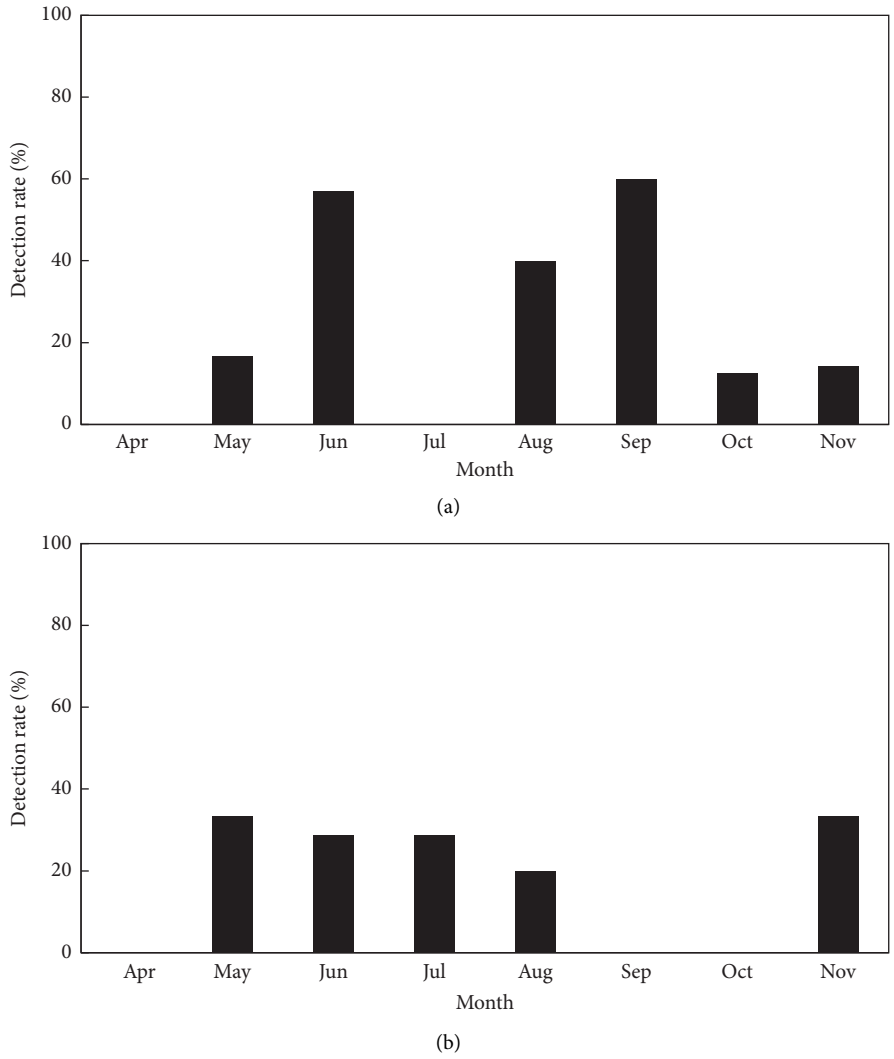

Figure 2: Monthly distributions of *Escherichia coli* in water samples (a) and aquatic animals (b) collected from aquaculture farms along the Korean coast in 2018.

detected in July, during which the water temperature was generally high (Figure 2(a) and Supplementary Table 1). The detection rate was relatively low (0–16.7%) in April, May, October, and November, consistent with a generally low temperature season in Korea. In our previous study, we reported that the monthly mean water temperature varied from $13.9 \pm 0.5^{\circ}$C to $26.4 \pm 0.5^{\circ}$C at stations 1–4 [18]. The temperatures were higher during the summer, with the highest temperature recorded in August (26.4°C), then in June (25.2°C). Collectively, although these results indicated that the prevalence of *E. coli* was generally high in the summer season (with the exception of July), they did not show a strictly positive association with water temperature.

3.2. Antimicrobial Resistance Patterns of E. coli. Table 2 and Supplementary Tables 5 and 6 show the antimicrobial resistance patterns of *E. coli* isolates ($n = 289$) from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Among the 289 isolates of *E. coli*, 74 isolates (25.6%) were resistant to TET; this was the highest resistance among all 15 antibiotic agents tested in this study. More than 20.0% of the isolates were resistant to 4 antibiotics, including STR (23.2%), CL (22.1%), CHL (21.8%), and SXT (21.8%). In contrast, more than 80.0% of the isolates were sensitive to 9 antimicrobials (GEN, AMC, MEM, FEP, CAZ, FIS, AMP, CIP, and NA); >76% of the isolates were sensitive to second-, third-, and fourth-generation cephalosporins (e.g., FOX, CAZ, and FEP); and >90% of the isolates were susceptible to quinolones (CIP and NA).

Overall, the findings indicated that *E. coli* isolates from aquaculture farms generally had low resistance to the broad spectrum of antibiotics tested in the present study. In addition, although there were some differences in antimicrobial resistance between *E. coli* from water samples and aquatic animals, the resistance patterns did not significantly differ (Supplementary Tables 5 and 6). Although antimicrobial resistance patterns vary among countries, resistance to TET, AMP, STR, and SXT are more prevalent than resistance to other antibiotics [26]. In our study, resistance to TET (25.6%), STR (23.2%), CHL (21.8%), and SXT (21.8%) were also prevalent in *E. coli* strains.

Table 2: Antimicrobial resistance of *Escherichia coli* isolates ($n = 289$) collected from water samples and aquatic animals from aquaculture farms along the Korean coast in 2018.

Antimicrobials	*Number (%) of isolates*		
	Susceptible	Intermediate	Resistant
Aminoglycosides			
Gentamicin (GEN)	249 (86.2)	0 (0)	40 (13.8)
Streptomycin (STR)	222 (76.8)	0 (0)	67 (23.2)
β-lactam/β-lactamase inhibitor combinations			
Amoxicillin/clavulanic acid (AMC)	235 (81.3)	4 (1.4)	50 (17.3)
Carbapenems			
Meropenem (MEM)	254 (87.9)	29 (10.0)	6 (2.1)
Cephems			
Cefepime (FEP)	246 (85.1)	1 (0.4)	42 (14.5)
Cefoxitin (FOX)	222 (76.8)	14 (4.9)	53 (18.3)
Ceftazidime (CAZ)	224 (84.4)	4 (1.4)	41 (14.2)
Folate pathway inhibitors			
Trimethoprim-sulphamethoxazole (SXT)	230 (79.6)	0 (0)	59 (20.4)
Sulfisoxazole (FIS)	246 (85.1)	0 (0)	43 (14.9)
Penicillins			
Ampicillin (AMP)	237 (82.0)	6 (2.1)	46 (15.9)
Phenicols			
Chloramphenicol (CHL)	209 (72.3)	17 (5.9)	63 (21.8)
Polymyxin			
Colistin (CL)	225 (77.9)	0 (0)	64 (22.1)
Quinolones			
Ciprofloxacin (CIP)	261 (90.3)	11 (3.8)	17 (5.9)
Nalidixic acid (NA)	271 (93.8)	0 (0)	18 (6.2)
Tetracyclines			
Tetracyclin (TET)	213 (73.7)	2 (0.7)	74 (25.6)

Antibiotics are widely used in aquaculture and livestock production [14, 27]. Enteric bacteria (including *E. coli*) are becoming increasingly resistant to currently available antimicrobials [28]. Notably, the TET antibiotic family is most frequently used in Korean aquaculture; 70 tons were used in 2019 [29]. In the present study, 25.6% of *E. coli* isolates from aquatic animals (fish and shrimp), and surrounding water exhibited resistance to TET; this rate was the highest among the tested antimicrobial agents. The previous study similarly demonstrated a high prevalence of *E. coli* resistance to TET in sardines and shrimps from Algeria [10]. In another study [12], *E. coli* isolates from major inland pollution sources (13.7%) and oysters (11.8%) in Korea had lower rates of TET resistance than did the isolates (25.6%) in the present study. Also, TET resistance (9.5%) of bacterial isolates from Nile tilapia farms in Egypt [30] was lower than that of *E. coli* isolates from the Korean aquaculture farms in this study.

The antibiotic CL is extensively used in agricultural production and as the last line of defense against critical infections caused by multidrug-resistant pathogens [15, 31, 32]. In our previous study [18], approximately 80% of *V. parahaemolyticus* isolates were highly resistant to CL, whereas the *E. coli* isolates in the present study had low resistance to CL. In Korea, CL is not used in aquaculture, although it is commonly used for livestock (cattle, pigs, and poultry); approximately, 10.5 tons were used in 2019 [29].

Table 3 shows the MAR index values for the *E. coli* isolates from the seawater samples and aquatic animals. The MAR index, first suggested by Krumperman [24] in a report concerning *E. coli*, is used to determine potential human health risks. MAR index values of >0.2 indicate that the source has a high risk of antimicrobial contamination. The MAR values ranged from 0.00 to 0.80; the highest value was for 11 isolates (3.8%) that were resistant to 12 antimicrobials. Most *E. coli* isolates (63.0%) were not resistant to the antibiotics tested. However, among the 289 *E. coli* isolates, 30.1% (87 isolates) showed a MAR value of 0.2, indicating resistance to at least three antimicrobials. In another study [10], all *E. coli* strains isolated from sardines and shrimps in Algeria exhibited multidrug resistance to antibiotic agents tested. Taken together, these findings indicate that multi-antibiotic resistance is highly prevalent in *E. coli*.

3.3. Antimicrobial Resistance Patterns of Enterococcus Species. Table 4, Figure 3, and Supplementary Tables 7 and 8 show the antimicrobial resistance patterns of *Enterococcus* species isolates ($n = 75$), including *E. faecium* ($n = 65$) and *E. faecalis* ($n = 10$), from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Among the 75 isolates of *Enterococcus* spp., 24 isolates (32.0%) were resistant to CIP (the highest resistance among all 12 antibiotic agents tested); this was followed by resistance to DAP (16.0%), TET (10.7%), ERY (6.7%), and SYN (2.7%) (Table 4). Numerous isolates showed intermediate resistance to ERY (93.3%) and SYN (64.0%). Sixteen (21.3%), 13 (17.3%), and 1 (1.3%) isolates were also intermediately resistant to LNZ, CIP, and CHL, respectively. In contrast, all

Table 3: Patterns and indexes of multiantibiotic resistance (MAR) of *Escherichia coli* isolates ($n = 289$) collected from water samples and aquatic animals from aquaculture farms along the Korean coast in 2018.

Number of antibiotics	*Number (%) of isolates resistant to antibiotic agents*						MAR index
	Water		*Animals*		*Total*		
	Intermediate	Resistant	Intermediate	Resistant	Intermediate	Resistant	
0	124 (75.1)	94 (57.0)	95 (76.6)	88 (71.0)	219 (75.8)	182 (63.0)	0.00
1	28 (17.0)	2 (1.2)	25 (20.2)	4 (3.2)	53 (18.3)	6 (2.1)	0.07
2	12 (7.3)	7 (4.3)	4 (3.2)	7 (5.7)	16 (5.5)	14 (4.8)	0.13
3	1 (0.6)	11 (6.7)	0	4 (3.2)	1 (0.4)	15 (5.2)	0.20
4	0	11 (6.7)	0	0	0	11 (3.8)	0.27
5	0	4 (2.4)	0	1 (0.8)	0	5 (1.7)	0.33
6	0	1 (0.6)	0	1 (0.8)	0	2 (0.7)	0.40
7	0	15 (9.1)	0	0	0	15 (5.2)	0.47
8	0	4 (2.4)	0	0	0	4 (1.4)	0.53
9	0	1 (0.6)	0	1 (0.8)	0	2 (0.7)	0.60
10	0	4 (2.4)	0	2 (1.6)	0	6 (2.1)	0.67
11	0	6 (3.6)	0	10 (8.1)	0	16 (5.5)	0.73
12	0	5 (3.0)	0	6 (4.8)	0	11 (3.8)	0.80
Total	165 (100)	165 (100)	124 (100)	124 (100)	289 (100)	289 (100)	

Table 4: Antimicrobial resistance of *Enterococcus* spp. isolates ($n = 75$) collected from water samples and aquatic animals from aquaculture farms along the Korean coast in 2018.

Antimicrobials	*Number (%) of isolates*		
	Susceptible	Intermediate	Resistant
Aminoglycosides			
Gentamicin (GEN)	75 (100)	0 (0)	0 (0)
Streptomycin (STR)	75 (100)	0 (0)	0 (0)
Glycopeptides			
Vancomycin (VAN)	75 (100)	0 (0)	0 (0)
Glycylcyclines			
Tigecycline (TGC)	75 (100)	0 (0)	0 (0)
Lipopeptides			
Daptomycin (DAP)	63 (84.0)	0 (0)	12 (16.0)
Macrolides			
Erythromycin (ERY)	0 (0)	70 (93.3)	5 (6.7)
Oxazolidinones			
Linezolid (LNZ)	59 (78.7)	16 (21.3)	0 (0)
Penicillins			
Ampicillin (AMP)	75 (100)	0 (0)	0 (0)
Phenicols			
Chloramphenicol (CHL)	74 (98.7)	1 (1.3)	0 (0)
Quinolones			
Ciprofloxacin (CIP)	38 (50.7)	13 (17.3)	24 (32.0)
Streptogramins			
Quinupristin/ Dalfopristin (SYN)	25 (33.3)	48 (64.0)	2 (2.7)
Tetracyclines			
Tetracyclin (TET)	67 (89.3)	0 (0)	8 (10.7)

Enterococcus spp. isolates were sensitive to 5 of the 12 antimicrobials used in this study, including GEN, STR, VAN, TGC, and AMP. More than 80.0% of the isolates were also susceptible to 3 agents (DAP, CHL, and TET), whereas no isolates were sensitive to ERY.

Of the 65 *E. faecium* isolates from water and animal samples, 36.9% were resistant to CIP (the highest resistance among all 12 antibiotic agents tested); this was followed by resistance to DAP (18.5%), ERY (7.7%), and TET (1.5%) (Figure 3(a) and Supplementary Table 7). In addition, a high percentage of the isolates exhibited intermediate resistance to ERY (92.3%) and SYN (63.1%). Sixteen (24.6%), 11 (16.9%), and 1 (1.5%) isolates were also intermediately resistant to LNZ, CIP, and CHL, respectively. In contrast, all *E. faecium* isolates were sensitive to 5 of the 12 antimicrobials used, including GEN, STR, VAN, TGC, and AMP; >80.0% of the isolates were also susceptible to 3 antimicrobial agents (DAP, CHL, and TET). Among the 10 *E. faecalis* isolates tested in this study, 7 (70.0%) and 2 (20.0%) isolates were resistant to TET and SYN, respectively (Figure 3(b) and Supplementary Table 8). All *E. faecalis* isolates showed intermediate resistance to ERY; the isolates also exhibited intermediate resistance to SYN (70.0%) and CIP (20.0%). In contrast, all isolates were susceptible to 8 of the 12 antimicrobial agents tested (GEN, STR, VAN, TGC, DAP, LNZ, AMP, and CHL); 80.0% of the isolates were sensitive to CIP. These results demonstrate that *E. faecium* showed resistance to more types of antibiotic agents than did *E. faecalis*.

Although *E. faecalis* exhibited very high resistance (70.0%) to TET, *E. faecium* exhibited very high sensitivity (98.5%) to TET, which belongs to the TET antibiotic family commonly used for aquaculture and livestock in Korea [29]. Notably, of the 8 isolates of Enterococci that were identified as TET-resistant, 7 were *E. faecalis*. These results indicated that TET should not be used for the clinical treatment of *E. faecalis* infections, particularly in Korea. Another study, also conducted in Korea, confirmed the high prevalence of TET-resistant*Enterococcus* species (*E. faecalis* and *E. faecium*) in retail raw meats (beef, pork, and chicken) and in sashimi (raw fish) [1].

VAN is often used to treat infections caused by enterococci. However, there have been reports of enterococci resistant to this drug; these have been designated as VAN-resistant enterococci [33]. Koluman et al. [33] demonstrated that 22% of *Enterococcus* spp. strains from different types of

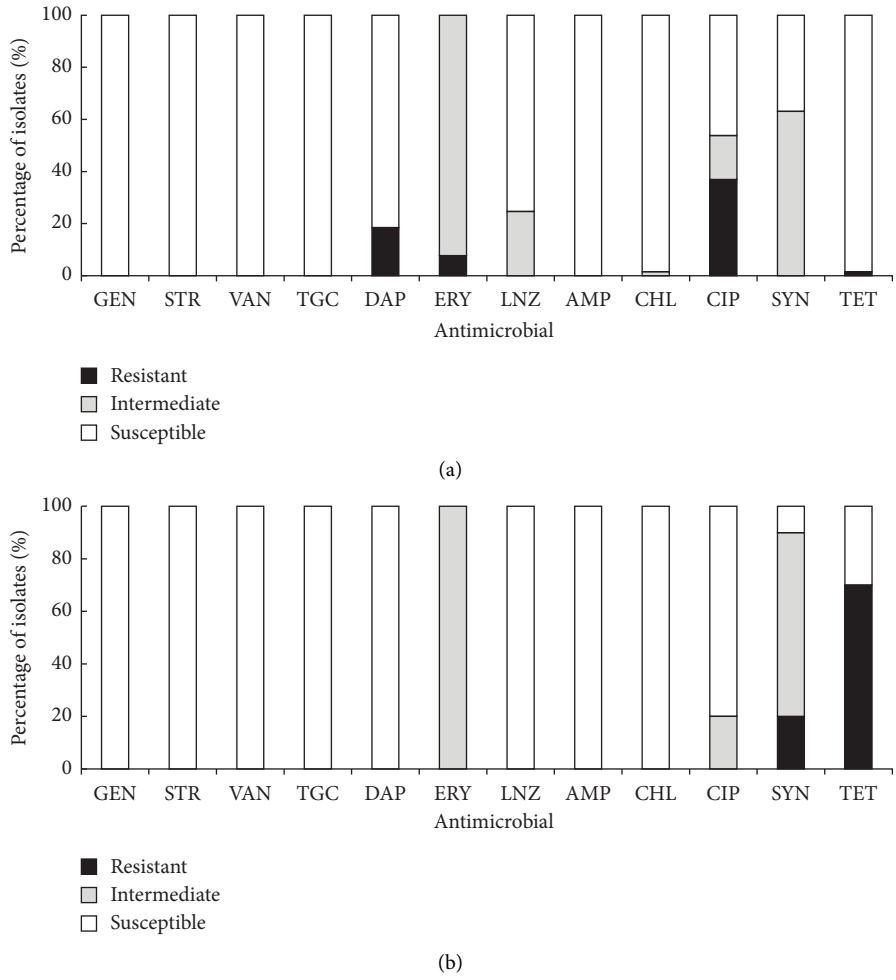

Figure 3: Antimicrobial resistance of *Enterococcus faecium* (a) and *Enterococcus faecalis* (b) isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018.

Table 5: Patterns and indexes of multiple-antibiotic resistance (MAR) of *Enterococcus faecium* isolates ($n = 65$) collected from water samples and aquatic animals from aquaculture farms along the Korean coast in 2018.

Number of antibiotics	*Number (%) of isolates resistant to antibiotic agents*						MAR index
	Water		*Animals*		*Total*		
	Intermediate	Resistant	Intermediate	Resistant	Intermediate	Resistant	
0	1 (4.2)	0	0	27 (65.9)	1 (1.5)	27 (41.5)	0.00
1	20 (83.3)	21 (87.5)	0	13 (31.7)	30 (30.8)	34 (52.3)	0.08
2	3 (12.5)	3 (12.5)	26 (63.4)	1 (2.4)	29 (44.6)	4 (6.2)	0.17
3	0	0	9 (22.0)	0	9 (13.9)	0	0.25
4	0	0	6 (14.6)	0	6 (9.2)	0	0.33
Total	24 (100)	24 (100)	41 (100)	41 (100)	65 (100)	65 (100)	

All *E. faecalis* isolates ($n = 10$) were resistant to less than one agent.

food in Turkey were resistant to VAN. Robredo et al. [34] also reported that 25 of 92 chicken samples were contaminated with VAN-resistant enterococci. Fortunately, in the present study, all strains from aquaculture farms were sensitive to VAN. Furthermore, sashimi (raw fish) and raw livestock products (beef, pork, and chicken) in Korea did not contain any VAN-resistant strains [1].

The MAR index values for *Enterococcus* spp. isolates from the seawater samples and aquatic animals are shown in Table 5. The MAR index values for *E. faecium* isolates ranged from 0.00 to 0.17. Moreover, the MAR index values for the isolates exhibiting intermediate resistance ranged from 0.00 to 0.33; the highest value was for six isolates that showed intermediate resistance to four of the antimicrobials tested.

Most *E. faecium* strains (75.4%) had MAR index values between 0.08 and 0.17, indicating that the strains were intermediately resistant to one or two types of antibiotics. Notably, of the 65 *E. faecium* isolates from water and aquatic animals, 23.1% (15 isolates) had a MAR value >0.2, indicating multiantibiotic intermediate resistance to at least three antimicrobials.

4. Conclusions

Fecal indicators are used to determine the hygienic quality of water and food products. They are used to determine whether the contamination can negatively affect the sanitary status of aquatic products farmed in coastal regions. *E. coli* was the most dominant among the fecal-associated microorganisms tested in this study. Of the 289 *E. coli* isolates, more than 80.0% were sensitive to 9 of 15 antimicrobials tested, including GEN, AMC, MEM, FEP, CAZ, FIS, AMP, CIP, and NA. In contrast, 25.6% of the *E. coli* isolates were resistant to TET, which is commonly used for aquaculture and livestock in Korea. More than 20.0% of the *E. coli* isolates were also resistant to 4 antibiotics (STR, CL, CHL, and SXT). Among the 75 isolates of *Enterococcus* spp., 24 isolates (32.0%) were resistant to CIP, followed by DAP (16.0%), TET (10.7%), ERY (6.7%), and SYN (2.7%). These findings indicated that fecal-indicator bacteria (including *E. coli, E. faecium,* and *E. faecalis*) from aquaculture farms along the Korean coast generally showed low resistance to the antibiotics tested in the present study. Notably, 30.1% of the *E. coli* isolates showed multiantibiotic resistance to at least three antimicrobials; *Enterococcus* spp. did not show a similar rate of multiantibiotic resistance. The results of this study provide important baseline data regarding the antimicrobial resistance of fecal-indicator bacteria isolated from limited marine environments. Moreover, the frequent presence of multiresistant *E. coli* strains in farmed aquatic animals is particularly problematic in Korea because raw seafood is commonly consumed as a part of Korean culture; therefore, ongoing *E. coli* surveillance is warranted to protect human health.

Data Availability

The data supporting the findings of this study are available within the article and the supplementary material file.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Soon Bum Shin was involved in methodology and formal analysis and wrote the original draft. Sung Rae Cho was involved in investigation, resources, validation, and data curation. Jong Soo Mok was involved in conceptualization and project administration and reviewed and edited the article. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This work was supported by a grant from the National Institute of Fisheries Science in Korea (R2023054). This manuscript has been posted on a preprint site [35].

Supplementary Materials

Supplementary Table 1: Monthly distributions of *Escherichia coli* isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 2: Stationary distributions of *Escherichia coli* isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 3: Monthly distributions of *Enterococcus* spp. isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 4: Stationary distributions of *Enterococcus* spp. isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 5: Antimicrobial resistance of *Escherichia coli* isolated from water samples collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 6: Antimicrobial resistance of *Escherichia coli* isolated from aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 7: Antimicrobial resistance of *Enterococcus faecium* isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. Supplementary Table 8: Antimicrobial resistance of *Enterococcus faecalis* isolated from water samples and aquatic animals collected from aquaculture farms along the Korean coast in 2018. (*Supplementary Materials*)

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