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Antimicrobial Resistance and Virulence Genes Presence in *Escherichia coli* Strains Isolated from Gomso Bay, Korea

Kwon-Sam Park*

Department of Food Science and Biotechnology, College of Ocean Science and Technology, Kunsan National University, Gunsan 573-701, Korea

Abstract

In total, 131 *Escherichia coli* isolates from surface seawater of the Gomso Bay, of Korea, were analyzed for their susceptibility to 22 different antimicrobials and for genes associated with antimicrobial resistance and virulence. According to the disk diffusion susceptibility test, the resistance to tetracycline was most prevalent (33.6%), followed by that to ampicillin (22.1%), ticarcillin (22.1%), and trimethoprim (16.8%). More than 46.6% of the isolates were resistant to at least one antimicrobial, and 22.9% were resistant to three or more classes of antimicrobials; these were consequently defined as multidrug resistant. We further found that 29 ampicillin-resistant isolates possessed genes encoding TEM-type (93.1%) and SHV-type (6.9%) β-lactamases. Among the 44 tetracycline-resistant isolates, *tetA* and *tetC* were found in 35 (79.5%) and 19 (43.2%), respectively, whereas *tetB* was detected in only three isolates (6.8%). With regard to virulence genes, merely 0.8% (n = 1) and 2.3% (n = 3) of the isolates were positive for the enteroaggregative *E. coli*-associated plasmid (*pCVD432*) gene and the enteropathogenic *E. coli*-specific attaching and effacing (*eae*) gene, respectively. Overall, these results not only provide novel insight into the necessity for seawater sanitation in Gomso Bay, but they help reduce the risk of contamination of antimicrobial-resistant bacteria.

Key words: Antimicrobial resistance, *Escherichia coli*, Gomso Bay, Virulence genes

Introduction

Escherichia coli is a facultative anaerobic bacterium found in the normal flora of the intestinal tract of humans and animals; in contrast, it has also been implicated in infectious diseases (Rosas et al., 2006). Currently, *E. coli* are widely used as a sanitation indicator of microbiological contamination in water and food (Lang et al., 1999). While, *E. coli* is harmless in general, certain virulent strains are common causes of infectious diarrhea and other enteric diseases (Clements et al., 2012). Currently, the following *E. coli* pathotypes have been recognized to cause diarrhea in humans: EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; STEC, Shiga toxin-producing *E. coli*; EAEC, enteroaggregative *E. coli*; ETEC, enterotoxigenic *E. coli*; and DAEC, diffusely adhering *E. coli* (Turner et al., 2006).

For livestock farming, antimicrobial agents are abundantly used not only for medical treatment and prophylaxis of bacterial infections but also in the animals' food to promote growth, which has resulted in an increase in antimicrobial-resistant bacterial strains (Aarestrup, 2005; Sapkota et al., 2008). The critical factor in the development of antimicrobial resistance is the ability of the bacteria to acquire and disseminate exogenous genes via mobile genetic elements, such as plasmids, transposons, DNA insertion elements, and genomic islands (Rowe-Magnus et al., 2002). Horizontal gene transfer is one of the most common mechanisms, by which antimicrobial resistance traits are transferred from one organism to another

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***Corresponding Author**
E-mail: parkks@kunsan.ac.kr

(Martinez, 2009). Since the discovery of integrons, a unique mechanism for the dissemination of antibiotic resistance genes (Stokes and Hall, 1989), the increase in bacterial strains resistant to several antimicrobial agents has raised public health concerns about treatment options, heath care costs, and foodborne illness (Hammerum and Heuer, 2009). Food contamination of antimicrobial-resistant bacteria could be a major public health threat, due to the possibility that the responsible genes, which are carried on mobile genetic elements, may be transferred to other bacteria that confer high risks to human health (Rowe-Magnus et al., 2002). *E. coli* strains, in particular, are candidate vehicles for such genetic transfers, not only due to their diversity but also because they exist as common microflora of the gastrointestinal tracts of humans and animals (Clements et al., 2012).

In Korea, numerous studies have focused on antimicrobial resistance patterns and genes in *E. coli* strains isolated from various sources, including humans, food and animals (You et al., 2006; Koo and Woo, 2011; Ryu et al., 2012a). However, very few of these studies provided adequate data regarding the prevalence of *E. coli* in seawater or seafood.

In this study, *E. coli* strains isolated from surface seawater of Gomso Bay, on the west coast of Korea, were characterized for their prevalence, antimicrobial resistance, and virulence gene expression using the disk diffusion method and polymerase chain reaction (PCR).

Materials and Methods

E. coli isolation and identification

In total, 131 environmental strains of *E. coli* were isolated from the surface seawater of Gomso Bay, Jeollabuk-do, Korea, between May and December of 2012. Seawater samples collected from sampling stations were transported within 4 h to the laboratory in ice-cold containers. The number of total coliform and fecal coliform bacteria was determined using the most probable number (MPN) method (American Public Health Association, 1970). Seawater was inoculated with lauryl tryptose broth (Difco; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and incubated for 48 ± 2 h at 35°C. One loop from each gas-positive tube was inoculated with Brilliant Green Bile Broth (Difco) and EC medium (Difco), and incubated for 48 ± 2 h at 35°C or 24 ± 2 h at 44.5 ± 0.2°C. Each gas-positive EC tube was plated onto eosin methylene blue agar (Difco) and incubated for 24 h at 35°C. One *E. coli* isolate per each seawater sample was randomly selected and validated using the API 20E Kit (bioMerieux, Marcy-l'Etoile, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing against 22 antimicrobials was performed on 131 *E. coli* isolates using the disk diffusion method on Mueller Hinton agar plates (Difco), according to the standards and interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) (2010). The following 22 antibiotics were used: amikacin (30 μg), amoxicillin (20 μg), ampicillin (10 μg), cefepime (30 μg), cefotetan (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), imipenem (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), norfloxacin (10 μg), rifampin (5 μg), streptomycin (10 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), tetracycline (30 μg), ticarcillin (75 μg), and trimethoprim (5 μg). Antimicrobial susceptibility test discs were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK), and the results were classified as susceptible, intermediate, or resistant according to the zone diameter interpretive standards recommended by CLSI (2010). The quality-control strains, *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, were included in each run.

PCR amplification of antimicrobial resistance and virulence genes

The presence of genes associated with tetracycline (*tetA*, *tetB*, and *tetC*) or β-lactams (TEM, SHV, and OXA) in 44 tetracycline-resistant or 29 ampicillin-resistant isolates, respectively, were detected using PCR. All isolates were also evaluated by PCR for the following genes: heat labile (*lt*), heat stable (*st*), attaching and effacing (*eae*), EAEC-associated plasmid (*pCVD432*), invasion-associated locus (*ial*), and Shiga toxin (*stx1* and *stx2*) genes. PCR was performed using the primer sets listed in Table 1. Genomic DNA was extracted from overnight bacterial cultures using the boiling method. After cooling, samples were microcentrifuged to remove cell debris, and the resulting supernatants were used as PCR templates. In each PCR reaction, 3 μL of the respective DNA template was suspended in 22 μL reaction mixtures containing 2.5 μL of 10× *Ex Taq* buffer (Takara, Kyoto, Japan), 50 pmol of each primer, 2 μL of dNTPs (2.5 mM of each dNTP), and 2 units of *Ex Taq* DNA polymerase (5 U/μL). PCR amplification of tetracycline-associated genes was conducted under the following conditions: initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. PCR amplification of β-lactam-associated genes was conducted under the following conditions: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 15 s, and extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. PCR conditions for detecting virulence genes consisted of initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min. All PCR reactions were performed using the

Gene Amp PCR System 9700 (Perkin Elmer, Norwick, CT, USA) and the amplified products were electrophoresed on 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

Results

Isolation of *E. coli* strains from surface seawater in Gomso Bay

A sanitary survey in an important shellfish-growing area of Gomso Bay, west coast of Korea, was conducted to evaluate bay conditions and compliance with bacteriological criteria for shellfish production. Seawater samples were collected 12 times from May to December 2012 from 20 sampling stations established in the survey area (Fig. 1). The values determined by the MPN-method for both the total coliform bacteria and fecal coliform bacteria in the 240 seawater samples ranged from less than 1.8 to 240 MPN/100 mL, respectively (Table 2). The geometric means and estimated 90th percentile values for total coliform bacteria ranged from 2.1 to 6.3 MPN/100 mL, and from 3.3 to 38.8 MPN/100 mL, respectively, whereas those for fecal coliform bacteria ranged from 1.9 to 4.1 MPN/100 mL, and from 2.7 to 18.9 MPN/100 mL, respectively. Notably, the levels of total coliform and fecal coliform bacteria from stations near the wastewater discharge sites (stations 3, 14, 18, 19, and 20) were slightly higher than those from other sites. The bacteria levels after rainfall were also higher, than those in

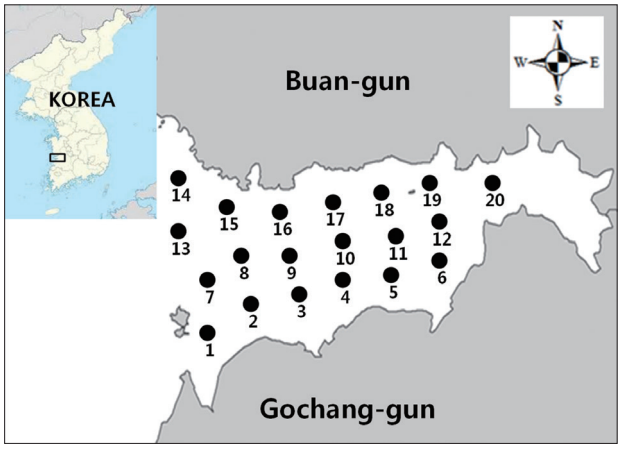

Fig. 1. Location of sample collection stations in Gomso Bay, Republic of Korea, from May to December 2012.

Table 1. Oligonucleotide primers used in this study

Gene	Primer	Nucleotide sequence (5′ to 3′)	Size (bp)	Reference
tetA	*tet*(A)-F	GTGAAACCCAACATACCCC	887	Van et al., 2008
	tet(A)-R	GAAGGCAAGCAGGATGTAG		
tetB	*tet*(B)-F	CCTTATCATGCCAGTCTTGC	773	Van et al., 2008
	tet(B)-R	ACTGCCGTTTTTTCGCC		
tetC	*tet*(C)-F	ACTTGCAGCCACTATCGAC	880	Van et al., 2008
	tet(C)-R	CTACAATCCATGCCAACCC		
TEM	TEM-F	GAGTATTCAACATTTTCGT	857	Van et al., 2008
	TEM-R	ACCAATGCTTAATCAGTGA		
SHV	SHV-F	TCGCCTGTGTATTATCTCCC	768	Van et al., 2008
	SHV-R	CGCAGATAAATCACCACAATG		
OXA	OXA-F	GCAGCGCCAGTGCATCAAC	198	Van et al., 2008
	OXA-R	CCGCATCAAATGCCATAAGCG		
estA	ST-F	GCTAAACCAGTAGGGTCTTCAAAA	147	Talukdar et al., 2013
	ST-R	CCCGGTACAGGCAGGATTACAACA		
eltB	LT-F	CACACGGAGCTCCTCAGTC	508	Talukdar et al., 2013
	LT-R	CCCCCAGCCTAGCTTAGTTT		
eae	eae-F	CCCGAATTCGGCACAAGCATAAGC	881	Talukdar et al., 2013
	eae-R	CCCGGATCCGTCTCGCCAGTATTCG		
aat	pCVD432-F	CTGGCGAAAGACTGTATCAT	650	Talukdar et al., 2013
	pCVD432-R	CAATGTATAGAAATCCGCTGTT		
stx1	stx1F	CACAATCAGGCGTCGCCAGCGCACTTGCT	606	Talukdar et al., 2013
	stx1R	TGTTGCAGGGATCAGTGGTACGGGGATGC		
stx2	stx2F	CCACATCGGTGTCTGTTATTAACCACACC	372	Talukdar et al., 2013
	stx2R	GCAGAACTGCTCTGGATGCATCTCTGGTC		
iaa	ial upper	CTGGATGGTATGGTGAGG	320	Talukdar et al., 2013
	ial lowe	GGAGGCCAACAATTATTTCC		

the dry season. Based on these data, the bacteriological quality of seawater in Gomso Bay met the criteria recommended by the National Shellfish Sanitation Program (Food and Drug Administration, 2005) and the Korea Shellfish Sanitation Program for shellfish production (Ministry of Marine Affairs and Fisheries, 2006). One strain from each seawater sample was isolated to further analyze antimicrobial resistance and virulence genes in isolated *E. coli* strains.

Antimicrobial susceptibility of *E. coli* isolates

In total, 131 isolates were tested for resistance against 22 antimicrobial agents (Table 3). Resistance to tetracycline (33.6%), ampicillin (22.1%), ticarcillin (22.1%), trimethoprim (16.8%), sulfamethoxazole/trimethoprim (16.0%), nalidixic acid (13.7%), streptomycin (11.5%), chloramphenicol (9.9%), kanamycin (7.6%), norfloxacin (6.1%), ciprofloxacin (2.3%), erythromycin (2.3%), and gentamicin (0.8%) was observed. These results are in agreement with several previous studies (You et al., 2006; Van et al., 2008; Koo and Woo, 2012; Ryu et al., 2012b). However, no isolate showed resistance to amikacin, amoxicillin, cefepime, cefotetan, cefoxitin, ceftriaxone, cephalothin, imipenem, or rifampin. Table 4 shows the resistance patterns of these isolates. Among the 131 *E. coli* isolates, 70 (53.4%) were not resistant to any of the 22 antimicrobial agents tested, whereas 61 (46.6%) were resistant to at least one antimicrobial agent. In total, 22.9% of the isolates were resistant to three or more classes of antimicrobials and defined as multidrug resistant. One strain, in particular, exhibited resistance to 11 antimicrobial agents (AMP, C, CIP, K, NA, NOR, S, SXT, TE, TIC, and TMP).

Characterization of antimicrobial resistance genes in *E. coli* isolates

Ampicillin-resistant isolates ($n = 29$) were investigated for the presence of β-lactamase-encoding genes using PCR with the primer sets specified in Table 1. We found that 27 isolates (93.1%) contained genes encoding TEM-type β-lactamases, whereas the rest had genes associated with SHV-type β-lactamases (data not shown), consistent with previous findings indicating that the former genes were among the most prevalent in ampicillin-resistant *E. coli* isolates derived from food and livestock (Van et al., 2008; Aslam et al., 2009; Ryu

Table 2. Summary of bacteriological examination results of each sampling stations in Gomso Bay, from May to December 2012

Station	MPN/100mL										No. of samples
	Total coliform					Fecal coliform					
	Range	GM[1)]	90th[2)]	>230		Range	GM[1)]	90th[2)]	>43		
				No.	%				No.	%	
1	<1.8-7.8	2.1	4.0	0	0.0	<1.8-4.6	1.9	2.7	0	0.0	12
2	<1.8-79	3.6	17.5	0	0.0	<1.8-13	2.5	6.3	0	0.0	12
3	<1.8-79	3.6	18.5	0	0.0	<1.8-49	3.0	11.2	1	8.3	12
4	<1.8-33	4.3	25.0	0	0.0	<1.8-23	3.2	11.4	0	0.0	12
5	<1.8-33	3.5	15.2	0	0.0	<1.8-13	2.8	7.6	0	0.0	12
6	<1.8-79	4.1	23.6	0	0.0	<1.8-13	3.0	8.8	0	0.0	12
7	<1.8-33	3.0	11.0	0	0.0	<1.8-13	2.4	5.5	0	0.0	12
8	<1.8-33	3.5	14.8	0	0.0	<1.8-33	3.3	13.7	0	0.0	12
9	<1.8-33	3.4	12.5	0	0.0	<1.8-13	2.7	6.9	0	0.0	12
10	<1.8-23	2.5	7.1	0	0.0	<1.8-7.8	2.3	4.8	0	0.0	12
11	<1.8-23	2.7	8.2	0	0.0	<1.8-13	2.4	5.7	0	0.0	12
12	<1.8-13	2.7	7.3	0	0.0	<1.8-7.8	2.3	4.8	0	0.0	12
13	<1.8-4.6	2.1	3.3	0	0.0	<1.8-4.5	1.9	2.7	0	0.0	12
14	<1.8-49	4.8	33.4	0	0.0	<1.8-49	2.9	12.7	0	0.0	12
15	<1.8-130	6.3	38.8	0	0.0	<1.8-23	4.1	15.0	0	0.0	12
16	<1.8-13	3.2	9.4	0	0.0	<1.8-7.8	2.3	4.8	0	0.0	12
17	<1.8-13	2.5	6.3	0	0.0	<1.8-4.5	2.2	3.9	0	0.0	12
18	<1.8-240	3.2	21.0	1	8.3	<1.8-240	2.9	17.8	1	8.3	12
19	<1.8-240	3.3	25.6	1	8.3	<1.8-240	3.0	18.9	1	8.3	12
20	<1.8-240	3.6	27.4	1	8.3	<1.8-130	3.0	16.2	1	8.3	12
Total	<1.8-240	3.4	16.5	3	1.3	<1.8-240	2.7	9.1	4	1.7	240

[1)]geometric mean. [2)]The estimated 90th percentile.

et al., 2012b).

Among the 44 tetracycline-resistant *E. coli* isolates, the *tetA* and *tetC* genes were found in 35 (79.5%) and 19 (43.2%) isolates, respectively, and *tetB* was found in only 3 (6.8%). Fourteen isolates (31.8%) tested positive for both *tetA* and *tetC*. Our results also showed that three tetracycline-resistant isolates possessed none of the tetracycline resistance genes analyzed (*tetA*, *tetB*, or *tetC*) (data not shown). These findings were consistent with previous studies demonstrating that genes encoding active efflux pumps of tetracycline, such as *tetA-E* and *tetG*, occurred frequently among tetracycline-resistant *E. coli* isolates derived from livestock and aquaculture environments (Van et al., 2008; Aslam et al., 2009; Tang et al., 2011; Koo and Woo, 2012; Ryu et al., 2012b).

Detection of virulence genes by PCR

All 131 isolates were also examined for the presence of seven virulence genes (*lt*, *st*, *eae*, *pCVD432*, *ial*, *stx1*, and *stx2*) using PCR. Approximately 3.1% (n = 4) of the isolates were positive for at least one of the seven virulence genes specific to certain *E. coli* pathotypes. Only 0.8% (n = 1) and 2.3% (n = 3) of the isolates were positive for the EAEC-associated plasmid (*pCVD432*) and the EPEC-specific attaching and effacing (*eae*) genes, respectively (data not shown). However, the STEC-associated *stx1* and *stx2* virulence genes, the ETEC-specific heat-stable toxin (ST) and heat-labile toxin (LT) genes, and the EIEC-specific invasion-associated locus (*ial*) gene were not detected. These results imply that none of the isolates were of the STEC, ETEC, or EIEC pathotype. The virulence gene frequency in the 131 isolates of this study was lower than that reported previously (Ram et al., 2008; Van et al., 2008; Talukdar et al., 2013).

Discussion

Our current study focused primarily on the prevalence of *E. coli* in Gomso Bay as well as the characterization of antimicrobial resistance and virulence genes in these isolates. Gomso Bay, connected to the Yellow Sea, is located between Gochang-gun and Buan-gun in Jeollabuk-do, Korea, covering approximately 76 km^2 with an average width, length, and depth of 5-6 km, 17 km, and 2-3 m, respectively. The bay is

Table 3. Antimicrobial resistance of *Escherichia coli* isolated from surface seawater in Gomso Bay

Antimicrobials	No. of isolates		
	Resistant	Intermediate	Susceptible
Amikacin	0	78	53
Amoxicillin	0	0	131
Ampicillin	29	37	65
Cefepime	0	10	121
Cefotetan	0	2	129
Cefoxitin	0	0	131
Ceftriaxone	0	0	131
Cephalothin	0	80	51
Chloramphenicol	13	23	95
Ciprofloxacin	3	16	112
Erythromycin	3	128	0
Gentamicin	1	30	100
Imipenem	0	0	131
Kanamycin	10	89	32
Nalidixic acid	18	60	53
Norfloxacin	8	30	93
Rifampin	0	108	23
Streptomycin	15	110	6
Sulfamethoxazole/ Trimethoprim	21	43	67
Tetracycline	44	28	59
Ticarcillin	29	3	99
Trimethoprim	22	29	80

Table 4. Antimicrobial resistance patterns of *Escherichia coli* isolated from surface seawater in Gomso Bay

	Resistance type	No. of resistant strains
1	TE	17
2	C	4
3	NA	4
4	AMP	1
5	TIC	1
6	TE-C	1
7	TE-S	1
8	AMP-TIC	1
9	TMP-SXT	1
10	TE-AMP-TIC	1
11	TMP-SXT-S	1
12	TE-AMP-TIC-S	4
13	TE-AMP-TIC-C	1
14	TE-TMP-SXT-K	1
15	AMP-TIC-TMP-SXT	1
16	TE-AMP-TIC-NA-K	1
17	TE-AMP-TIC-NA-S	1
18	TE-AMP-TIC-S-C	1
19	AMP-TIC-TMP-SXT-S	1
20	TE-TIC-TMP-SXT-S-C	1
21	TE-AMP-TMP-SXT-S-C	1
22	TE-AMP-TIC-TMP-NA-S	1
23	AMP-TIC-TMP-SXT-K-C	1
24	TE-AMP-TIC-TMP-SXT-NA	2
25	TE-AMP-TIC-TMP-SXT-K	1
26	TE-AMP-TIC-TMP-SXT-S-C	1
27	AMP-TIC-TMP-SXT-NA-K-NOR	1
28	TE-AMP-TIC-TMP-SXT-NA-NOR	2
29	TE-AMP-TIC-TMP-SXT-NA-GM	1
30	TE-AMP-TIC-TMP-SXT-NA-K-NOR-S	1
31	TE-AMP-TIC-TMP-SXT-NA-K-NOR-C-E	1
32	TE-AMP-TIC-TMP-SXT-NA-K-NOR-CIP-E	2
33	TE-AMP-TIC-TMP-SXT-NA-K-NOR-CIP-C-S	1
Total		61

a source of shellfish and other aquaculture organisms. Waste from agriculture, household and stock breeding of numerous small rivers that flow into Gomso Bay are thought to be the current source of contaminants in the area.

Virulent or antimicrobial-resistant bacteria in drainage water can contaminate shellfish and fisheries in the bay and potentially be hazardous for human health. Many antibiotics are used for therapeutic treatment and prophylactic purposes in animals and humans; consequently, increase in antimicrobial resistance due to excess use and misuse of antimicrobials is a public health concern (World Health Organization, 2001). Therefore, antimicrobial usage in animal feed should be tightly regulated to decrease the prevalence of antimicrobial-resistant bacteria.

In this study, 131 environmental *E. coli* strains isolated from surface seawater of Gomso Bay were tested for resistance against 22 antimicrobial agents. Tetracycline resistance was most prevalent, followed by that to ampicillin, ticarcillin, trimethoprim, sulfamethoxazole/trimethoprim, nalidixic acid, and streptomycin. Of the 61 isolates displaying resistance to at least 1 antimicrobial, resistance patterns varied and were classified among 33 types (Table 4).

Ampicillin resistance in gram-negative bacteria is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thereby inactivate antibiotics (Massova and Mobashery, 1998). In many genera of gram-negative bacteria, the most predominant β-lactamases are TEM-, SHV-, CTX-, and OXA-type enzymes (Bradford, 2001). TEM-1, a plasmid-mediated β-lactamase, is the most common β-lactamase in gram-negative bacteria, and accounts for up to 90% of the ampicillin resistance in *E. coli* (Livermore, 1995). In the present study, we found the TEM-type β-lactamases to be the most prevalent among 29 ampicillin-resistant isolates, consistent with previous studies (Livermore, 1995; Van et al., 2008; Aslam et al., 2009; Ryu et al., 2012b). In addition, both the *tetA* and *tetC* genes prevailed among the 44 tetracycline-resistant *E. coli* isolates. In the analyses of virulence genes, only 3.1% of the isolates were positive for the EAEC-associated plasmid (*pCVD432*) and the EPEC-specific attaching and effacing (*eae*) genes.

E. coli is a facultative anaerobic bacterium found in the normal intestinal flora in humans and animals, however, some toxic strains can induce pathogenicity ranging from mild diarrhea to fatal complications (Tannock, 1995; Clements et al., 2012). While we did not investigate the mechanisms underlying the development of antimicrobial resistance in these *E. coli* isolates, several previous studies have established a causal relationship between antimicrobial use in aquaculture and the increase in specific antimicrobial-resistant bacterial strains (Schmidt et al., 2001; Petersen et al., 2002; Ribeiro et al., 2010; Ye et al., 2013).

In conclusion, continuous surveillance of antimicrobial-resistant bacteria in Gomso Bay is necessary to ensure the safety of fisheries and shellfish production.

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