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Antimicrobial Susceptibility Studies

Molecular characterization of class 1 integrons and antimicrobial resistance in *Aeromonas* strains from foodborne outbreak-suspect samples and environmental sources in Taiwan

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Abstract

One hundred thirty-three *Aeromonas* spp. isolates were examined for multiple antibiotic resistance phenotypes and prevalence of class 1 integron sequences. Twenty-four (18.0%) of these isolates contained class 1 integron. Seven different class 1 integrons were found among 24 strains, with a total of 10 different gene cassettes encoding for resistance to trimethoprim (*dfr12* and *dfr2d*), aminoglycosides (*aadA1* and *aadA2*), β-lactam antibiotics (*oxa2*), chloramphenicol (*catB3* and *catB8*), quaternary ammonium amines (*qacE2*), and 2 ORFs (*orfD* and *orfF*) with unknown function. Rate of antibiotic resistance was different between integron-positive and integron-negative strains. Trimethoprim and trimethoprim–sulphamethoxazole resistances were commonly associated with integron, and all of integron-positive isolates were multiple resistant to more than 3 agents. Resistance to as many as 10 antimicrobial agents were observed in integron-positive strains. Several cassette arrays of class 1 integrons identified in this study were not previously reported in *Aeromonas* strains. This study demonstrates the wide distribution of class 1 integron in *Aeromonas* spp. isolated from foodborne outbreak-suspect samples and environmental sources in Taiwan.

Keywords: *Aeromonas* spp.; Antibiotic resistance; Integron; Multiple resistance

1. Introduction

Aeromonads are widespread in natural habitats, and some species cause both gastrointestinal and extraintestinal infectious diseases in human (Kirov, 1997). *Aeromonas* gastroenteritis is generally self-limiting, and antibiotic treatment is unnecessary. However, for extraintestinal infections, the susceptibility patterns should be known to implement appropriate therapy (Vila et al., 2002). Reports from many parts of the world suggest that members of aeromonads readily develop single or multiple antibiotic resistances (Ko et al., 1996; Goñi-Urriza et al., 2000; Schmidt et al., 2001; Palú et al., 2006). The emergence and dissemination of antibiotic-resistant bacteria are a serious concern worldwide. The dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria, and acquisition of antibiotic resistance genes is facilitated by mobile genetic elements, such as integrons (Ploy et al., 2000). Integrons have been found to play an important role in the carriage and dissemination of antibiotic resistance genes (Fluit and Schmitz, 1999). Integrons are able to capture one or more gene cassettes from the environment and incorporate them by using site-specific recombination. The role of integrons and gene cassettes in the dissemination of multidrug resistance in Gram-negative bacteria is well established (Hall and Stokes, 1993). Based on the sequences of integrase

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genes, at least 8 different classes of integrons have been described in Gram-negative bacteria (Nield et al., 2001).

Class 1 integrons are most commonly found in clinical isolates of Gram-negative bacteria and are strongly associated with multiple antibiotic resistances (Bass et al., 1999; Goldstein et al., 2001). These elements contribute to the spread of genetic determinants of antimicrobial resistance by horizontal gene transfer; although not mobile themselves, they are frequently associated to plasmids and transposons (Fluit and Schmitz, 1999; Martinez-Freijo et al., 1999). Class 1 integrons carry integrase gene (*intI1*), which code for the site-specific recombinase responsible for cassette insertion (Martinez and de la Cruz, 1990; Collis et al., 1993), and include the *attI1* site where the cassettes are integrated and a promoter, P_c, is responsible for the transcription of the cassette-encoded genes (Collis and Hall, 1995; Partridge et al., 2000). Gene cassette contains a single antibiotic resistance gene and a 59-base element (or *attC* site) downstream of the gene, which is responsible for recombination events (Collis et al., 2002).

Data on antimicrobial resistance in *Aeromonas* spp. are commonly obtained from clinical isolates of human, food, and environmental samples (Neyts et al., 2000; Radu et al., 2003) because they are important vehicles of dissemination of this pathogen. However, only a few studies have, to date, addressed the prevalence of class 1 integrons among environmental bacteria (Rosser and Young, 1999; Schmidt et al., 2001). In this study, we have determined the antimicrobial resistance rates and analyzed the class 1 integron population in strains of *Aeromonas* isolates from foodborne outbreak-suspect samples and environmental sources in Taiwan.

2. Materials and methods

2.1. Bacterial isolates and antimicrobial susceptibility

A total of 133 *Aeromonas* spp. were obtained from 3 sources: foodborne outbreak-suspect samples ($n = 80$), aquatic animal ($n = 26$), and poultry ($n = 27$). Of these, 50 were *Aeromonas caviae*, 45 *Aeromonas hydrophila*, 31 *Aeromonas veronii* bv. sobria (otherwise known as *A. sobria*), and 6 *Aeromonas encheleia*, and 1 was *A. veronii* bv. veronii. Disc diffusion method for antibiotic susceptibility testing was conducted as described by Vivekanandhan et al. (2002). The *Aeromonas* strains were tested against the following antibiotic discs (Oxoid, Basingstoke, Hampshire, United Kingdom): amikacin (30 μg), ampicillin (10 μg), cefazolin (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), sulphamethoxazole (25 μg), tetracycline (30 μg), trimethoprim (5 μg), and trimethoprim–sulphamethoxazole (1:19) (25 μg). After enrichment in Brain Heart Infusion Broth at 35 °C for 6 to 8 h, the cultures were streaked on Mueller–Hinton agar plates using a cotton swab. With an antibiotic disc dispenser (Oxoid, Basingstoke, Hampshire, United Kingdom), the discs were placed on the agar surface. After 30 min of prediffusion time, the plates were incubated at 35 °C for 24 h before the inhibition zones were measured and compared with the susceptible control strain, *Escherichia coli* ATCC 25922, whose susceptibility pattern was as expected. All the tests were performed in duplicates, and when results were different, the experiment was repeated to resolve the discrepancy. The zones of growth inhibition were evaluated according to the Clinical and Laboratory Standards Institute (CLSI, 2006). Statistical significance (P value) was calculated using Pearson χ^2 test in terms of number of resistance/intermediate resistance strains and susceptible strains in the integron-positive and integron-negative groups.

2.2. DNA extraction and polymerase chain reaction amplification of integrase genes

The strains were cultured in trypticase soy broth at 37 °C for 24 h. After harvest, the DNA was extracted using Puregene DNA Isolation Kit (Model D6000A; Gentra System, Minneapolis, MN). To determine whether the *Aeromonas* isolates carry integrons, we used polymerase chain reaction (PCR) amplification to detect class 1 and class 2 integrase genes, *intI1* (IntI1-F/-R) and *intI2* (IntI2-F/-R), respectively (Mazel et al., 2000). The primers used for the amplification of these genes and the predicted sizes of the amplification products are listed in Table 1. DNA samples (5 ng per reaction mixture) were amplified in a 25 μL reaction mixture consisting of 50 mmol/L potassium chloride, 10 mmol/L Tris chloride (pH 8.3), 1.25 mmol/L magnesium chloride, 200 μmol/L (each) deoxyribonucleotide triphosphate, 2.0 μmol/L primers, and 1.5 U of FastStart *Taq* DNA

Table 1
Primers used for PCR analysis of integrons

Primer	Nucleotide sequence (5′ to 3′)	Target	Product size (bp)	Reference
IntI1-F	GGGTCAAGGATCTGGATTTCG	*intI1*	483	Mazel et al., 2000
IntI1-R	ACATGGGTGTAAATCATCGTC			
IntI2-F	CACGGATATGCGACAAAAAGGT	*intI2*	788	Mazel et al., 2000
IntI2-R	GTAGCAAACGAGTGACGAAATG			
CalI-F	TTATGGAGCAGCAACGATGT	Class 1 integron	Variable	This study
CalI-R	CTGTGAGCAATTATGTGCTT	Variable region		
A16SF	GGGAGTGCCTTCGGGAATCAGA	16S rRNA	356	Wang et al., 2003
A16SR	TCACCGCAACATTCTGATTTG			

polymerase (Roche Diagnostic, Mannheim, Germany). Amplifications were performed with a model 9700 DNA thermal cycler (Applied Biosystems, Foster City, CA). Conditions for amplification using the IntI1 and IntI2 primers were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s; 62 °C for 30 s; and 72 °C for 60 s. Conditions for amplification using the Cal1 primers were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 120 s. If no amplification of the targeted gene was observed, then amplification of a 356 bp fragment of 16S rRNA sequence was used as a positive control and distilled water (without DNA template) as a negative control. The sequence of the primers and the PCR operating conditions for the 16S rDNA are as described by Wang et al. (2003).

2.3. Amplification and sequencing of gene cassette regions

The gene cassette regions for class 1 integrons were amplified with primer pairs Cal1-F/Cal1-R. To determine whether different isolates carry identical gene cassette, we characterized the cassette genes of every isolate. That is, each cassette gene PCR amplification product was sequenced. To analyze the sequences of the gene cassette regions of the integrons, we cloned the PCR products into pCRII-TOPO vector (Invitrogen, Carlsbad, CA) and transformed it into *E. coli* DH5α cells. Sequencing reactions were performed using a double-stranded plasmid preparation by dideoxy chain termination with T7 and SP6 primers. The nucleotide sequences of the integron gene of *Aeromonas* strains have been submitted to the GenBank nucleotide sequence database under accession numbers DQ462518, DQ462519, DQ462520, DQ515960, DQ519078, EF090608, and EF422367.

3. Results

Class 1 and class 2 integrons were analyzed in 133 *Aeromonas* strains, and PCR amplification was used to detect class 1 and class 2 integrase genes. Among the 133 isolates amplified, *intI1* gene cassette regions were amplified in 74 isolates, but none of the isolates was positive in PCR assays specific for the *intI2* gene. For the detection of class 1 integron cassette (variable regions) in all *intI1*-positive isolates, PCR was performed with primers (Cal1-F/Cal1-R) for the 5′ and 3′ conserved segments. Among the 74 isolates carrying *intI1*, 50 isolates were "empty" with no gene cassette inserted between the conserved segments of the integron. The other 24 isolates carried gene cassette in class 1 integrons; the sizes of these gene cassette regions ranged from 0.9 to 2.1 kb. Upon sequencing the entire integrons, 10 different gene cassettes were identified, including genes encoding for resistance to trimethoprim (*dfrA1*, *dfrA5*), β-lactam antibiotics (*oxa2*), chloramphenicol (*catB3*, *catB8*), and aminoglycosides (*aadA1*, *aadA2*); *qacE2* encoding a small protein that confers resistance to quaternary ammonium compounds; as

Table 2
Characteristics of the integrons-containing *Aeromonas* isolates

Strain no.	Species	Origin	Gene cassettes	GenBank accession no.	Size (bp)	Antibiotic resistance profile
170	*A. caviae*	F	*aadA1*	DQ462518	938	AM, KZ, RL, S, SXT, W
175	*A. caviae*	F	*aadA1*		938	AM, KZ, RL, TE, W
27	*A. veronii* bv. sobria	A	*qacE2*/*orfD*	DQ462520	939	AM, NA, RL, (S)[a], TE
28	*A. hydrophila*	A	*qacE2*/*orfD*		939	AM, (KZ), RL, (W)
40	*A. hydrophila*	A	*qacE2*/*orfD*		939	AM, KZ, RL, (S), W
43	*A. veronii* bv. sobria	A	*qacE2*/*orfD*		939	AM, KZ, (K), RL, (S), (W)
45	*A. caviae*	A	*qacE2*/*orfD*		939	AM, KZ, NA, RL, TE
50	*A. caviae*	F	*qacE2*/*orfD*		939	AM, KZ, RL
55	*A. caviae*	A	*qacE2*/*orfD*		939	AM, C, KZ, RL, TE
66	*A. veronii* bv. sobria	A	*qacE2*/*orfD*		939	AM, C, KZ, NA, RL, TE
114	*A. hydrophila*	F	bal(*oxa2*)/*orfD*	DQ519078	1278	AM, (KZ), RL
333	*A. hydrophila*	F	*catB8*/*aadA1*	EF090608	1653	AM, KZ, RL, S
701	*A. hydrophila*	P	*catB3*/*aadA1*	DQ462519	1653	AM, KZ, NA, RL,(S)
723	*A. caviae*	P	*catB3*/*aadA1*		1653	AM, KZ, NA, RL, TE
727	*A. hydrophila*	P	*catB3*/*aadA1*		1653	AM, KZ, NA, RL,(TE)
745	*A. hydrophila*	P	*catB3*/*aadA1*		1653	AM, KZ, NA, RL, TE, W
752	*A. veronii* bv. sobria	P	*catB3*/*aadA1*		1653	AM, (KZ), NA, RL,(S), TE, W,
758	*A. hydrophila*	P	*catB3*/*aadA1*		1653	AM, KZ, NA, RL, S, W,
38	*A. veronii* bv. sobria	A	*dfr12*/*orfF*/*aadA2*	DQ515960	1842	AM, C, K, NA, RL, S, SXT, (TE), W
67	*A. veronii* bv. sobria	A	*dfr12*/*orfF*/*aadA2*		1842	AM, C, K, NA, RL, S, TE, SXT, W,
74	*A. veronii* bv. sobria	A	*dfr12*/*orfF*/*aadA2*		1842	AM, C, K, KZ, NA, RL, S, SXT, TE, W
180	*A. caviae*	F	*dfr12*/*orfF*/*aadA2*		1842	AM, K, KZ, NA, RL, S, SXT, (TE), W
334	*A. hydrophila*	F	*dfr12*/*orfF*/*aadA2*		1842	AM, K, KZ, RL, SXT, TE, W
168	*A. encheleia*	F	*dfr2d*/*catB3*/*aadA1*	EF422367	2061	AM, C, KZ, NA, RL, (S), SXT, TE, W

F = foodborne outbreak-suspect samples; A = aquatic animal; P = poultry; AM = ampicillin; C = chloramphenicol; K = kanamycin; KZ = cefazolin; NA = nalidixic acid; RL = sulphamethoxazole; S = streptomycin; SXT = trimethoprim–sulphamethoxazole; TE = tetracycline; W = trimethoprim.

[a] Parentheses indicate intermediate resistance.

Table 3
Antibiotic susceptibility of integron-positive and integron-negative strains of *Aeromonas* spp.

Antibiotics (μg)[a]	Integron positive (n = 24)			Integron negative (n = 95)			P[b]
	% R	% I	% S	% R	% I	% S	
Kanamycin (30)	20.8	4.2	75.0	1.1	2.1	96.8	<0.05
Streptomycin (10)	29.2	25.0	45.8	1.1	14.7	84.2	<0.05
Cefazolin (30)	75.0	12.5	12.5	72.1	10.7	17.2	NS
Chloramphenicol (30)	25.0	0	75.0	2.1	2.1	95.8	<0.05
Tetracycline (30)	50.0	12.5	37.5	24.2	8.4	67.4	<0.05
Trimethoprim–sulphamethoxazole (25)	29.2	0	70.8	1.1	0	98.9	<0.05
Sulphamethoxazole (25)	100	0	0	69.5	1.1	29.5	<0.05
Trimethoprim (5)	50.0	8.3	41.7	6.3	3.2	90.5	<0.05
Nalidixic acid (30)	58.3	0	41.7	25.3	0	74.7	<0.05

NS = not statistically significant; R = resistance; I = intermediate resistance; S = susceptible.

[a] Concentration of disks.

[b] Statistical significance (P value) was calculated using Pearson χ^2 test in terms of number of resistance/intermediate resistance strains and susceptible strains in the integron-positive and integron-negative groups.

well as 2 different ORFs (*orfD* and *orfF*) of unknown function. The most commonly found genes among class 1 integron were *aadA* genes. Two *aadA* genes (*aadA1* and *aadA2*) were detected in 5 different class 1 integrons. Four class 1 integrons carried *aadA1* and one carried *aadA2*. Two *dfr* genes (*dfr2d* and *dfr12*) and 2 *cat* genes (*catB3* and *catB8*) were detected in 2 different class 1 integrons, respectively (Table 2).

Of the 24 amplified class 1 integron variable regions, 7 isolates were 1653 bp in size. Although inserted regions were indistinguishable with respect to size, nucleotide sequencing showed that variable regions contained 2 gene cassettes: one was identified as *aadA* gene, and the other gene cassette was identified as *cat* gene orthology (*catB3* and *catB8*). Similar results were also found in 10 isolates, which were about 940 bp in size; 8 isolates carried both *qacE2* and *orfD* (939 bp); and 2 isolates carried single *aadA1* (938 bp) (Table 2).

Antimicrobial resistance testing by disc diffusion was performed and evaluated according to the CLSI (2006). The susceptibility pattern for the integron containing strains is shown in Table 2, and the percentage of *Aeromonas* strains showing resistance against each antibiotic is given in Table 3. The data from integron-positive and integron-negative isolates were compared with relate integron carriage and resistance profile. All the strains were resistant to ampicillin followed by sulphamethoxazole and cefazolin with more than 70% of strains presenting full or intermediate resistance; however, all the tested strains were susceptible to amikacin, gentamicin, and ceftriaxone. The susceptibility to chloramphenicol, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole, tetracycline, trimethoprim, and trimethoprim–sulphamethoxazole varied in integron-positive and integron-negative isolates. In the integron-positive strains, except cefazolin, the frequency of resistance to most of antimicrobial agents was significantly ($P < 0.05$) higher than integron-negative strains (Table 3). All of integron-positive *Aeromonas* spp. were multidrug resistant (resistant to 3 or more commonly used antibiotics). Resistance to more than 9 antibiotics was observed in 5 strains (nos. 38, 67, 74, 168, and 180), of which 2 were isolated from foodborne sample and 3 from fish. Most of the integron-positive isolates displayed resistance to multiple antibiotics compared with the integron-negative isolates.

4. Discussion

Class 1 integron and gene cassettes have been found in a number of different bacterial genera and appear to be prevalent in nature (Goldstein et al., 2001). Of the 133 strains analyzed, class 1 integron was identified in 24 (18.0%) strains. This prevalence was higher than that reported by Rosser and Young (1999), who showed that 3.6% of Gram-negative bacteria in an estuarine environment contained the class 1 integron in UK. In contrast, other reports from clinical isolates revealed that the prevalence of class 1 integrons in other Gram-negative bacteria were 52% in Taiwan (*E. coli*) (Chang et al., 2000), 54.6% in Korea (*E. coli*) (Yu et al., 2003), 59% in France (Enterobacteriaceae) (Sallen et al., 1995), and 9.4% in China (*Shigella* spp.) (Pan et al., 2006). This dissimilarity may be due to the differences in bacterial genera or isolate sources. Despite the low prevalence in this study, high diversity of integrons was observed. Seven different class 1 integrons were detected, and all of the gene cassettes were described in other microorganisms (White and Rawlinson, 2001; Pai et al., 2003; Soler Bistue et al., 2006; Williams et al., 2006), indicating the high potential of these structures to be transferred within microorganisms because of their transportation within plasmids or conjugative transposons. Our results also support the hypothesis that integrons provide a very efficient strategy for the acquisition and dissemination of new antibiotic resistance genes.

In about 67.6% strains harboring *intI1*, gene cassette amplicon was not obtained or the amplicon size was less than 200 bp. This may be because the inserted gene cassette regions were too large to be amplified by conventional PCR techniques, or such integrons may lack the 3′ conserved segment generally associated with this class of integron

(Barlow et al., 2004), or amplification products of approximately 150 bp probably corresponded to empty structures (Bissonnette and Roy, 1992). Poole et al. (2006) also reported that roughly half of the integrons characterized did not contain integron-associated gene cassettes, and some of the integrons lacked the 3′ conserved region.

Although only 8 integron-containing strains isolated from foodborne samples were reported in this study, they contained 6 different integrons (Table 2). On the other hand, 6 strains isolated from different raw meats of poultry carry the same integron containing *catB3* and *aadA1* gene cassettes, and 1 strain (no. 333) isolated from foodborne sample carries the integron containing *catB8* and *aadA1* gene cassettes. However, integron carrying *dfr12*, *orfF*, and *aadA2* was found in *A. caviae* (no. 180), *A. veronii* bv. sobria (nos. 38, 67, and 74), and *A. hydrophila* (no. 334) isolated from foodborne and aquatic animal samples. An identical integron was reported in an *E. coli* isolate from the United States (Williams et al., 2006) and Taiwan (Chang et al., 2000), and in *Staphylococcus aureus* isolate from South China (Shi et al., 2006). These results reveal that the same integron can disseminate in different bacterial species or genera, in different sample sources, and in different areas of the world. The most frequently detected resistance genes were aminoglycoside adenyltransferase genes (*aadA1* and *aadA2*) that confer resistance to streptomycin and spectinomycin. This similar observation was reported for motile aeromonads isolated from bovine feces in Australia (Barlow et al., 2004) and Gram-negative bacteria isolated from aquatic environment (Rosser and Young, 1999).

Many classes of antimicrobial agents, such as aminoglycosides, chloramphenicol, tetracycline, and trimethoprim–sulphamethoxazole have been reported to be active against aeromonads (Clark, 1992; Koehler and Ashdown, 1993; Zhao et al., 2001). Multiple resistance phenotypes were observed in class 1 integron-containing isolates in our study (Table 2). Integron and gene cassettes have been identified as a primary source of resistance and are suspected to serve as reservoirs of antimicrobial resistance genes within microbial populations (Bass et al., 1999; Ochman et al., 2000). Trimethoprim combination with sulphamethoxazole is commonly used for the treatment of gastroenteritis (Zhao et al., 2001). Among the integron-positive isolates, significant increase in the number of strains resistant to trimethoprim and trimethoprim–sulphamethoxazole was observed. A high correlation between the presence of integrons carrying *dfr* gene and resistance to trimethoprim and trimethoprim–sulphamethoxazole was observed (Table 2). Trimethoprim is a broad-spectrum antimicrobial agent active against enteric pathogens, and trimethoprim alone or in combination with sulphamethoxazole has been commonly used to treat infection (Yu et al., 2004). Of the 133 tested strains, 7 isolates were trimethoprim–sulphamethoxazole resistant, and among these, 6 have an integron carrying *dfr* gene, 5 harboring *dfr12*, and 1 harboring *dfr2d*. This suggests that class 1 integrons are an important genetic element for resistance to trimethoprim and trimethoprim–sulphamethoxazole among *Aeromonas* isolates. Fifteen integron-positive isolates contained the *aadA1* or *aadA2*, but not all isolates are resistant to streptomycin. Resistance to streptomycin was not observed in most isolates that harbor *aadA1*, but resistance to the streptomycin (except no. 334) and the closely related kanamycin was seen in isolates containing *aadA2* in the integrons (Table 2). Similar observations were made in several previous reports. Pan et al. (2006) reported that resistance to streptomycin was not observed in *Shigella* spp. isolates harboring a class 1 integron that contains different *aadA* cassette. Roe et al. (2003) observed that 3 *E. coli* isolates, harboring a class 1 integron that contains a single cassette of *aadA1*, were resistant to kanamycin but not resistant to streptomycin. Conjugal transfer of plasmids harboring *aadA1* gene cassette to a strain of *Hafnia alvei* has been reported; however, the transfer did not confer resistance to streptomycin (Zhao et al., 2001), and it was suggested that expression of the inserted gene cassette by the integron promoter was inefficient. In our study, 7 of the 8 isolates carrying *catB* (chloramphenicol acetyltransferase) genes in integrons are sensitive to chloramphenicol; this may be due to the inefficient expression as reported by Zhao et al. (2001). Potrykus and Wegrzyn (2001) found low levels of acetyl coenzyme A in *cat*-expressing *E. coli* CM2555 in the presence of chloramphenicol, which led the bacterium to be sensitive to this antibiotic. The results indicate that integron-bearing strains may not always express the resistance to antibiotics. Furthermore, many isolates showed resistance to antibiotics but did not possess the corresponding antibiotic resistance gene cassettes within the integrons characterized from such isolates. Lee et al. (2001) reported that not all of the *dfrA1* genes were found to be associated with integron gene cassettes. It is suggested that such resistances are encoded by nonintegron elements. In our study also, 5 integron-positive strains (nos. 38, 55, 66, 67, and 74) were resistant to chloramphenicol, but *cat* cassettes were not found in the integron, implying the nonintegron sources of this gene. The antimicrobial phenotype probably relate to nonenzymatic chloramphenicol resistance (*cmlA*) gene or other antimicrobial mechanism. We also observed that the *dfr* gene cassettes are situated closer to the promoter region (5′ terminal) of the integron and therefore were probably expressed more efficiently then other gene cassettes.

In conclusion, this study demonstrated that class 1 integrons are an important genetic element for resistance to trimethoprim combination with sulphamethoxazole among *Aeromonas* isolates. These bacterial strains are potential reservoirs for antimicrobial resistance genes and play an important role in transfer of antibiotic resistance gene among bacterial populations in nature. To our knowledge, arrangement of some of the gene cassettes in this study was never reported in *Aeromonas* strains. However, a similar gene cassette array of *dfr2d*/*catB3*/*aadA1* found in *A. encheleia* (no. 168) had already been reported in *A. hydrophila* (GenBank accession number AY751518). All of these

10 gene cassettes found among *Aeromonas* spp. isolates in this study are also often present in clinical and environmental isolates of the Gram-negative bacteria. These findings suggest that the transfer of antibiotic resistance genes can occur through gene cassettes on class 1 integrons among *Aeromonas* spp. and other bacteria.

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