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Characterization of an IncA/C Multidrug Resistance Plasmid in *Vibrio alginolyticus*

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Cephalosporin-resistant *Vibrio alginolyticus* was first isolated from food products, with β-lactamases encoded by bla_{PER-1}, bla_{VEB-1}, and bla_{CMY-2} being the major mechanisms mediating their cephalosporin resistance. The complete sequence of a multidrug resistance plasmid, pVAS3-1, harboring the bla_{CMY-2} and *qnrVC4* genes was decoded in this study. Its backbone exhibited genetic homology to known IncA/C plasmids recoverable from members of the family *Enterobacteriaceae*, suggesting its possible origin in *Enterobacteriaceae*.

Vibrio alginolyticus, like other *Vibrionaceae* species, such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, may cause a range of human infections, such as gastroenteritis, septicemia, and necrotizing fasciitis. Of the pathogenic *Vibrio* species, *V. alginolyticus* is one of the top three causes of infections in humans and is genetically homologous to *V. parahaemolyticus* (1, 2). Resistance to expanded-spectrum cephalosporins and fluoroquinolones has also been reported in *V. parahaemolyticus* and *V. cholerae* but not in other *Vibrio* species such as *V. vulnificus* and *V. alginolyticus* (3, 4). Here we report (i) the isolation from food products of *V. alginolyticus* strains that are resistant to expanded-spectrum cephalosporins and (ii) the resistance mechanisms involved.

V. alginolyticus strains were isolated from food samples, including shrimp, chicken, pork, and beef, in Shenzhen, China, from June 2014 to August 2015 as previously described (5). Suspicious colonies from thiosulfate-citrate-bile salt sucrose and CHROMagar plates were screened by PCR and DNA sequencing and confirmed with API 20E test strips (bioMérieux, Inc.). A total of 23 *V. alginolyticus* strains were isolated from 515 food samples (2 from 88 chicken samples, 1 from 258 pork samples, 1 from 121 beef samples, and 19 from 48 shrimp samples). These 23 *V. alginolyticus* strains were subjected to antimicrobial susceptibility testing according to the Clinical and Laboratory Standards Institute (6). These isolates exhibited a high rate of resistance to ampicillin (100%), followed by trimethoprim-sulfamethoxazole (48%); ceftriaxone and cefotaxime (26%); chloramphenicol (13%); cefoxitin and amoxicillin-clavulanic acid (8.7%); and nalidixic acid, ciprofloxacin, ofloxacin, and gentamicin (4.4%). All strains were susceptible to tetracycline, amikacin, and meropenem. Five of six cephalosporin-resistant strains were isolated from shrimp samples in the same market on different dates, whereas one strain, VAS3-1, was isolated from a chicken sample (Table 1). Characterization of these isolates by pulsed-field gel electrophoresis (PFGE) was performed as previously described (7) and revealed that these isolates had distinct PFGE profiles, suggesting a high level of genetic diversity of these isolates even though most of them were recovered from the same market at different times (see Fig. S1 in the supplemental material). The prevalence of β-lactamase genes among the strains was determined by PCR assays as previously described (8) and showed that the bla_{PER-1} β-lactamase gene is present in strains VA1 and VA6 and that the bla_{CMY-2} gene is present in VAS3-1. The bla_{VEB-1} element was detected in strains V2 and V5, but no known β-lactamase gene was found in strain V4 (Table 1). It should be noted that this is the first time these cephalosporinases have been described in *V. alginolyticus* and that this is the first time the bla_{VEB-1} element has been recovered from a *Vibrio* species. Conjugation experiments performed with these strains showed that three strains, namely, VA1, VAS3-1, and VA5, could successfully transfer their cephalosporin resistance phenotypes to a recipient strain, *Escherichia coli* J53. Plasmid typing results showed that the conjugative plasmid recovered from VAS3-1 belonged to the IncA/C type, whereas conjugative plasmids from VA1 and VA5 were untypeable. Our laboratory has recently recovered an IncA/C-type conjugative plasmid from a *V. parahaemolyticus* strain (3). We therefore further characterized the IncA/C conjugative plasmid recovered from strain VAS3-1 in this study and investigated whether it is genetically related to the one obtained from *V. parahaemolyticus*. S1 PFGE and hybridization with a bla_{CMY-2} probe performed with VAS3-1 showed that the strain contains two bla_{CMY-2}-positive plasmids of ~180 and 300 kb, respectively, but only the ~180-kb plasmid was transferrable to the *E. coli* J53 recipient strain (see Fig. S2 in the supplemental mate-

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TABLE 1 Antibiotic susceptibility profiles of cephalosporin-resistant *V. alginolyticus* strains and corresponding recipient transconjugant *E. coli* strain J53

Strain	2015 isolation date	Source	β-Lactamase gene product	MIC (mg/liter)[b] of:										
				AMP	AMC	CTX	CRO	NAL	CIP	AMK	TET	CHL	GEN	SXT
J53				1	4/2	0.03	0.015	1	0.03	0.5	0.5	2	0.25	0.25/4.75
VA1	1/14	Shrimp	PER-1	>64	16/8	>16	>16	0.5	0.06	4	2	0.25	32	>16/304
T-VA1			PER-1	>64	8/4	>16	>16	4	0.015	0.5	8	2	32	16/304
VA2	1/20	Shrimp	VEB-1	>64	4/2	>16	>16	32	>16	1	8	32	4	>16/304
VAS3-1	3/14	Chicken	CMY-2	>64	64/32	8	16	16	0.5	0.5	16	32	1	>16/304
T-VAS3-1			CMY-2	>64	64/32	8	>16	8	0.5	0.5	8	16	1	8/152
VA4	3/4	Shrimp	ND[a]	>64	4/2	>16	>16	0.5	0.5	2	4	>32	4	8/152
VA-5	3/14	Shrimp	VEB-1	>64	8/4	>16	>16	2	1	4	0.5	1	8	0.25/4.75
T-VA5			VEB-1	>64	4/2	2	2	8	0.03	0.5	0.5	2	8	0.25/4.75
VA6	3/30	Shrimp	PER-1	>64	4/2	16	8	1	0.25	4	2	2	4	8/152

[a] ND, not detected.
[b] AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CRO, ceftriaxone; CTX, cefotaxime; NAL, nalidixic acid; CIP, ciprofloxacin; AMK, amikacin; TET, tetracycline; CHL, chloramphenicol; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole.

rial). This 180-kb conjugative plasmid is larger than the ~150-kb *V. parahaemolyticus* plasmid reported in our previous study (3).

The plasmid from a transconjugant, T-VAS3-1, was sequenced with the Illumina NextSeq 500 platform and the PacBio RSII single-molecule real-time sequencing platform. The completed plasmid sequence was confirmed by PCR and then annotated with the RAST tool and the NCBI Prokaryotic Genome Annotation Pipeline. The plasmid was designated pVAS3-1 and was found to be a circular IncA/C plasmid of 175,254 bp with a G+C content of 52.3%. It contained 216 predicted open reading frames, among which three core regions were disrupted by three resistance-encoding fragments (Fig. 1). The backbone exhibited a high degree of genetic similarity to six other known IncA/C plasmids (99% identity with 82 to 91% coverage) and was found to harbor genes responsible for plasmid replication, horizontal transfer, and maintenance of genetic stability (9, 10) (Fig. 2). All seven plasmids were bla_{CMY}-harboring IncA/C conjugative plasmids, and each

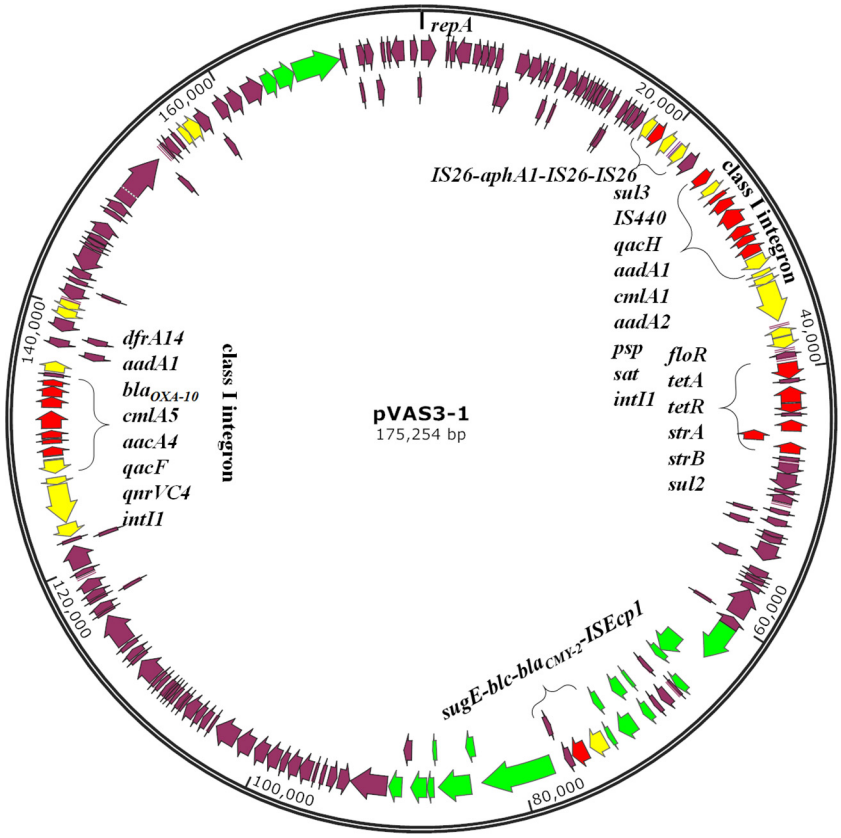

FIG 1 Gene map of conjugative IncA/C plasmid pVAS3-1. Yellow arrows represent mobile elements. Red arrows represent resistance genes. Blue arrows represent genes related to conjugal transfer functions.

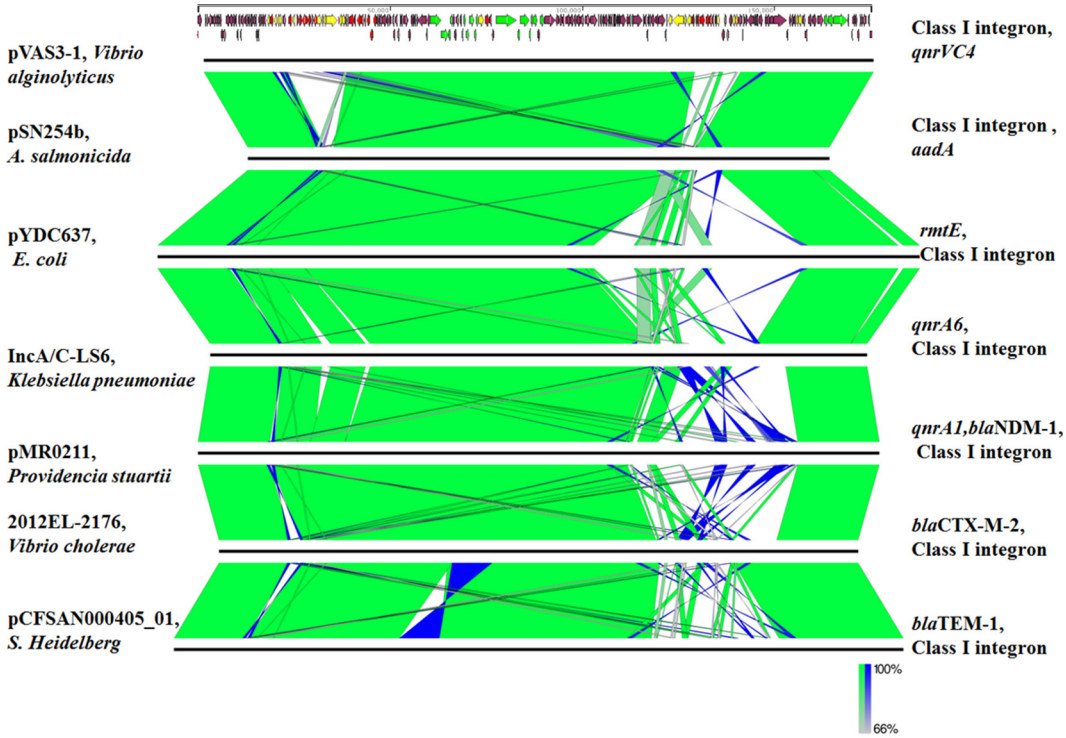

FIG 2 Whole-plasmid alignment of highly homologous bla_{CMY-2} IncA/C plasmids recovered from various bacterial species. Plasmid names and sources are on the left; elements located in variable mosaic regions are on the right. GenBank accession numbers: KJ909290, KP056256, JX442976, JN687470, CP007636, and KR091911.

contained a mobile mosaic multidrug resistance (MDR) region located between conserved backbone genes *ybaA* and *rhsD*. Presumably, with the aid of transposons and other mobile elements, different resistance determinants (*rmtE*, bla_{NDM-1}, the *mer* operon, etc.) were found incorporated into the plasmid backbone (9, 11). Resembling other bla_{CMY-2}-positive plasmids, the IS*Ecp1*-bla_{CMY-2} region was also found to be located between the *traA* and *traC* genes in pVAS3-1 (Fig. 1) (12). The bla_{CMY-2} gene was contained in a mobile element, IS*Ecp1*-bla_{CMY-2}-*blc-sugE*, that has previously been found mainly in members of the family *Enterobacteriaceae*, may originate from the *Citrobacter freundii* chromosome, and is transferrable to other bacterial species through IS*Ecp1*-mediated mobilization and conjugative plasmids (12, 13). It has also recently been recovered in IncA/C plasmids harbored by *Aeromonas salmonicida* and *Vibrio parahaemolyticus* (3, 14). In the region upstream of the conserved bla_{CMY-2} fragment, another conserved MDR region common to the seven plasmids that contained the *floR*, *tet*(A/R), *strA*, *strB*, and *sul2* elements was also detected. In addition, an IS*26*-*aphA1*-IS*26* fragment and a nonclassic class I integron without the 3′ coding sequence region were also found in the upstream region of pVAS3-1 (Fig. 1). This integron, which harbored six gene cassettes, was interrupted by the IS*440* and *sul3* elements. Such an integron structure was found in an *Enterobacteriaceae* species only once before (15). The presence of this unique integron in pVAS3-1 and not in the other six IncA/C plasmids suggests that pVAS3-1 may have originated from one of these six IncA/C plasmids (Fig. 2). Although some researchers have speculated that an aquatic environment may be the source of IncA/C plasmids (16), in view of the above data and the increasing reports of bla_{CMY-2}-positive plasmids in aquatic bacteria such as *Aeromonas* and *Vibrio* species (GenBank accession no. KJ909290 and CP007636) in recent years, we believe that dissemination of bla_{CMY-2} plasmids from *Enterobacteriaceae* to aquatic pathogens may be the most likely event.

The hypervariable region of pVAS3-1 was found to contain a ca. 20-kb MDR-encoding fragment, including the novel class I integron In*1222* (*qnrVC4*, *qacF*, *aacA4*, *cmlA5*, bla_{OXA-10}, *aadA1*, and *dfrA14*), a macrolide resistance determinant (*mphA-mrx-mphR*), and a mercury resistance operon. The *qnrVC4* gene was first identified in *Aeromonas punctata* in China in a classic class I integron (17). The class I integron found in pVAS3-1 is partially homologous to the one reported in *Salmonella enterica* serovar Rissen (18) in Thailand and different from the one reported in *A. punctata* (see Fig. S3 in the supplemental material). *qnrVC* alleles that encode quinolone resistance determinants that mediate decreased susceptibility to fluoroquinolones have emerged globally (17, 18). To date, seven different *qnrVC* alleles have been identified, mainly in the *Vibrionaceae* family; however, these resistance elements have also been recovered in other pathogens, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (7, 18, 19), highlighting the possibility that *qnrVC* alleles can be readily transmitted from aquatic-environment organisms to clinical pathogens.

In conclusion, comparative analysis of the genetic features of pVAS3-1 and plasmids recoverable from other bacterial species indicates that such elements exhibit a high degree of genetic plasticity and that the increasing reports of IncA/C plasmids in aquatic bacteria may be due to the transmission of these plasmids from *Enterobacteriaceae*.

Nucleotide sequence accession number. The sequence of plasmid pVAS3-1 has been deposited in GenBank under accession no. KU160531.

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