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Identification and Characterization of IncA/C Conjugative, bla_{NDM-1}-Bearing Plasmid in *Vibrio alginolyticus* of Food Origin

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Vibrio alginolyticus is a Gram-negative halophilic and mesophilic bacterium, particularly associated with severe epidemic vibriosis, which causes high mortality in marine animals, including fish, shellfish, and shrimp (1). The carbapenemase NDM-1 has been found mainly in *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella pneumoniae* (2). However, recent reports have described the existence of bla_{NDM} genes in *Vibrio parahaemolyticus*, *Vibrio fluvialis*, and *Vibrio cholerae* from clinical or environmental samples (3, 4). In this study, a *Vibrio* sp. strain, Vb1394, was isolated from a shrimp sample in a market of Shenzhen, China, in August 2016. The isolate was shown to be resistant to ceftriaxone, cefotaxime, amoxicillin-clavulanate, ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole (SXT); intermediate resistant to meropenem and imipenem; and susceptible to tetracycline, amikacin, gentamicin, nalidixic acid, and chloramphenicol (Table 1). The isolate was screened for the known β-lactamase genes by using previously described multiplex PCR assays and was shown to harbor bla_{NDM-1} (5). A conjugation experiment using azide-resistant *Escherichia coli* J53 as the recipient strain was performed to determine the transferability of the multidrug resistance property, with results showing that the carbapenem, cephalosporin, and SXT resistance phenotype could be transferred to *E. coli* J53. Interestingly, the transconjugant, designated TCVb1394, was highly resistant to meropenem and imipenem; however, the parental strain Vb1394 had reduced susceptibility to these two antibiotics in Mueller-Hinton (MH) medium without additional Zn^{2+}, although it carried bla_{NDM-1}, which generally referred to carbapenem resistance (Table 1). A similar observation was also reported from previous studies, in which carbapenems did not appear to offer any substantial activity against these NDM producers, and even a few isolates carrying NDM-1 had low MIC values, as low as 0.125 mg/liter (6); this warrants further investigation. S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) and hybridization were performed on the donor strain Vb1394 and the transconjugant TCVb1394 using the bla_{NDM-1} probe, with results confirming that bla_{NDM-1} was located on a plasmid with a size of ca. 165 kb, which could be transferred by conjugation. To describe the detailed genetic context of this plasmid, pC1394 was extracted from TCVb1394 and sequenced with the Illumina NextSeq 500 platform and the MinION sequencing platform, as the workflow reported previously (7).

Complete sequence analysis indicated that the bla_{NDM-1}-positive plasmid, designated pC1394 (GenBank accession number MH457126), was found to be a circular IncA/C-type plasmid of 167,140 bp, with 200 predicted coding sequences (CDSs), including a 128-kb backbone (65.5% GC content) and a single 39-kb resistance island (56.1% GC content). Based on the presence or absence of *orf1832-orf1847*, *rhs1-rhs2*, i1,

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TABLE 1 MICs of different antibiotics against *Vibrio alginolyticus* strain Vb1394 and its transconjugant TCVb1394 using azide-resistant *Escherichia coli* J53 as the recipient strain

Strain	MIC (mg/liter) by antibiotic[a]												
	CRO	CTX	AMC	AMP	MRP	IMI	TET	AMK	GEN	CIP	NAL	CHL	SXT
J53	0.03	0.03	4/2	8	0.03	0.25	2	1	0.25	0.015	4	4	0.25
Vb1394	>16	>16	>64/32	>64	2	2	2	2	4	16	1	1	8
TCVb1394	>16	>16	>64/32	>64	8	16	2	1	0.25	0.12	8	4	8

[a]CRO, ceftriaxone; CTX, cefotaxime; AMC, amoxicillin-clavulanic acid; Amp, ampicillin; MRP, meropenem; IMI, imipenem; TET, tetracycline; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; SXT, sulfamethoxazole-trimethoprim.

and i2, which were the key features distinguishing between type 1 and type 2 A/C_2 plasmids (8), pC1394 was considered to be a type 1 A/C_2 plasmid. Comparative analysis of pC1394 and pRMH760 showed that the major difference was located in the multiple-resistance region, designated ARI-A (Fig 1A). The ARI-A of pRMH760 contained six resistance genes, *aadB*, *sul1*, *dfrA10*, *aphA1*, *catA1*, and bla_{TEM-1}, clustered together in the region of about 45 kb, while that of pC1394 contained a novel complex class 1 integron carrying *dfrA15*, *aadA*, *sul1*, *ble*, bla_{NDM-1}, and *qnrA*. According to the previous report, genetic structures surrounding bla_{NDM-1} were commonly associated with the presence of IS*Aba125* (9). Notably, a composite transposon named Tn*125*, composed of two copies of insertion sequence IS*Aba125* bracketing a ca. 8-kb fragment (bla_{NDM-1}-*ble-trpF-tat-dct-groS-groL*-IS*CR27*), has been demonstrated to be responsible for the dissemination of bla_{NDM-1} in bacteria. A BLAST search of Tn*125* of pC1394 has identified a similar region in two other type 1 A/C_2 plasmids, pNDM-1_Dok01 (GenBank accession number AP012208) from *E. coli* and pNDM-KN (GenBank accession number JN157804)

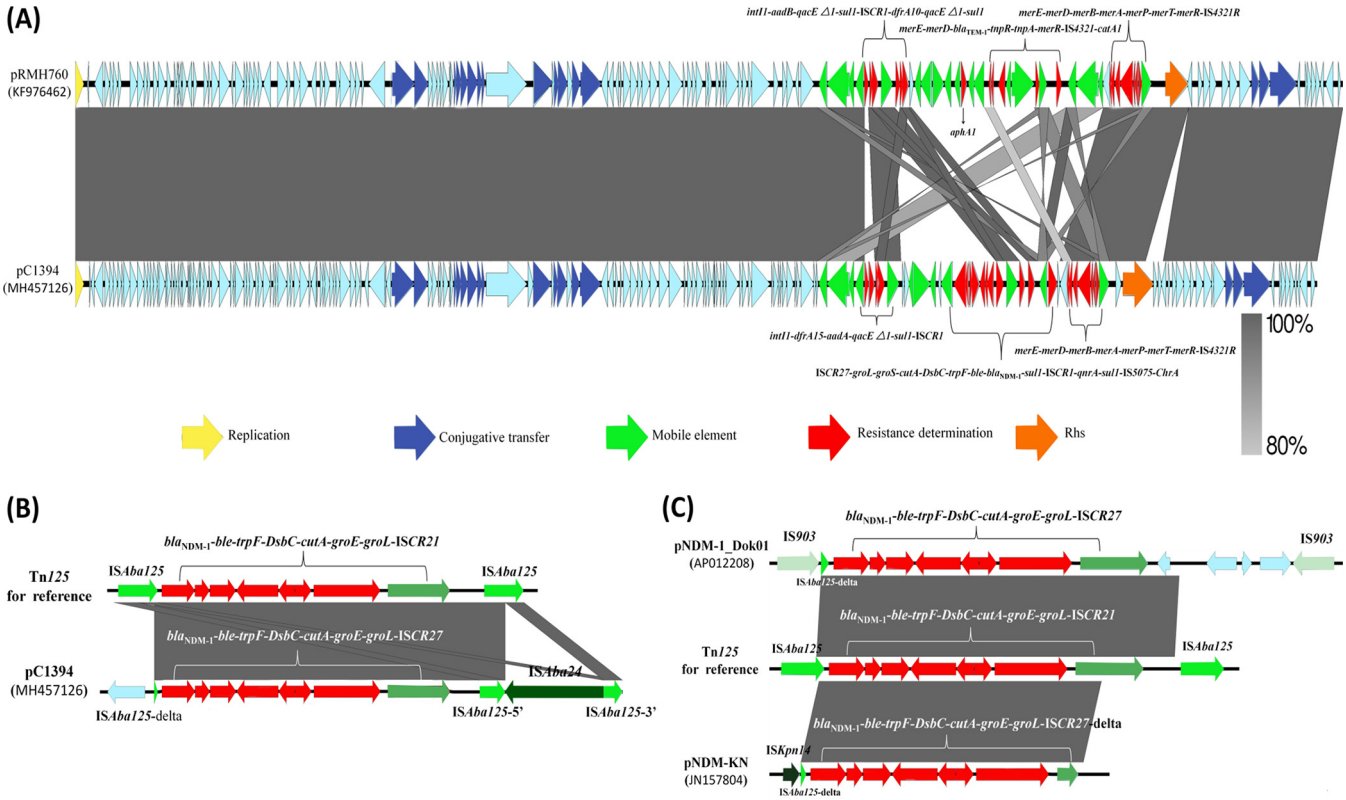

FIG 1 Comparative genetic analysis of pC1394 with other IncA/C_2 plasmids. (A) Comparison between pC1394 and pRMH760. Sequence similarity is depicted by the shaded region. Genes coding for proteins of known function are named above and colored according to the figure legend. (B and C) Comparison of ARI-A islands and mobile elements encoding the bla_{NDM-1} gene from different plasmids. Genes are denoted by arrows. Genes and mobile elements are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity, ≥99%). The GenBank accession numbers for each plasmid are as follows: KF976462 (pRMH760), MH457126 (pC1394), AP012208 (pNDM-1_Dok01), and JN157804 (pNDM-KN).

from *K. pneumoniae*. The Tn*125* from these three plasmids shared the same core structure of the bla_{NDM-1} mobile element, while different surrounding insertion sequence (IS) elements suggested their important role in the evolution and mobilization of the bla_{NDM-1} mobile element (Fig 1B and C). The initial mobilization of bla_{NDM-1} might occur through a transposition involving IS*CR27*, and then different mobile elements, such as IS*Aba125*, seem to have moved segments that contain bla_{NDM-1} into existing multidrug resistant (MDR) plasmids on type 1 A/C_2 plasmids. IncA/C plasmids are thought to be vehicles that have an extremely broad bacterial host range and may play an important role in the spread of bla_{NDM-1} in China (10). All three type 1 A/C_2 plasmids studied here harbor segments matching different parts of Tn*125*, suggesting that different mechanisms appear to be responsible for the independent transfer and further indicating a variation in the ways in which bla_{NDM-1} has been acquired by the same type plasmids.

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