

## Supplementary Materials

**Table S1.** The phenotypes of the four *V. cholerae* isolates used in this study.

<i>V. cholerae</i> Isolate	Source	Resistance		16S rRNA Gene (GeneBank Accession No.)
		Antibiotic	Heavy Metal	
7-6-5	<i>Aristichthys nobilis</i>	MXF/RIF	Hg <sup>2+</sup> /Ni <sup>2+</sup> /Pb <sup>2+</sup> /Zn <sup>2+</sup>	OR984968
L10-48	<i>Aristichthys nobilis</i>	AMP/STR	-	OR984969
L1-1	<i>Ctenopharyngodon idellus</i>	-	Hg <sup>2+</sup> / Pb <sup>2+</sup>	OR984970
B5-86	<i>Parabramis pekinensis</i>	STR	Hg <sup>2+</sup> / Pb <sup>2+</sup> /Zn <sup>2+</sup>	OR984971

Note: AMP, Ampicillin; STR, Streptomycin; RIF, Rifampicin; MXF, Moxifloxacin.

**Table S2.** The seventy-one *V. cholerae* strains with genome sequences used in the phylogenetic tree.

<i>V. cholerae</i> Isolate	Source	Collection Date	Serotype	Location	ST	GenBank Accession No.
1154-74	Homo sapiens	1974	O49	India	14	GCA_000969235.1
10432-62	Homo sapiens	1962	O27	Philippines	741	GCA_000969265.1
2559-78	Crab	1978	O1	USA	75	GCA_001857145.1
FC1877	Homo sapiens	1995	O139	India	69	GCA_002194155.1
FC3611a	Homo sapiens	1999	O139	India	69	GCA_002194165.1
FC3611b	Homo sapiens	1997	O139	India	69	GCA_002194185.1
FC2273	Homo sapiens	1998	O139	India	69	GCA_002194215.1
FC2271	Homo sapiens	1997	O139	India	69	GCA_002194235.1
FC1384	Homo sapiens	2000	O139	India	69	GCA_002194245.1
FC1341	Homo sapiens	2002	O139	India	69	GCA_002194265.1
FC1105	Homo sapiens	2003	O139	India	69	GCA_002194295.1
FC1817	Homo sapiens	1994	O139	India	69	GCA_002194305.1
FC1225	Homo sapiens	2001	O139	India	69	GCA_002194335.1
234-93	Homo sapiens	1993	O141	India	42	GCA_000737005.1
254-93	Homo sapiens	1993	O144	India	555	GCA_000737025.1
Vc306	Pork	2015	Non-O1/O139	China	Unknown	GCA_016456645.1
Vc3023	Pork	2015	Non-O1/O139	China	Unknown	GCA_016456685.1
Vc3022	Pork	2019	Non-O1/O139	China	Unknown	GCA_016456695.1
Vc382	Pork	2015	Non-O1/O139	China	Unknown	GCA_016456725.1
Vc3024	Pork	2019	Non-O1/O139	China	Unknown	GCA_016456735.1
Vc3021	Pork	2019	Non-O1/O139	China	Unknown	GCA_016456755.1
Vc226	Chicken	2015	Non-O1/O139	China	Unknown	GCA_016456785.1
Vc2634	Shrimp	2017	Non-O1/O139	China	Unknown	GCA_016456805.1
Vc2632	Shrimp	2017	Non-O1/O139	China	Unknown	GCA_016456815.1
Vc2633	Shrimp	2017	Non-O1/O139	China	Unknown	GCA_016456845.1
Vc1461	Chicken	2016	Non-O1/O139	China	Unknown	GCA_016456915.1
Vc601	Shrimp	2015	Non-O1/O139	China	Unknown	GCA_016456945.1
Vc730	Shrimp	2015	Non-O1/O139	China	Unknown	GCA_016456985.1
Vc401	Shrimp	2015	Non-O1/O139	China	Unknown	GCA_016456995.1
Vc518	Shrimp	2015	Non-O1/O139	China	Unknown	GCA_016457045.1
Vc515	Pork	2015	Non-O1/O139	China	Unknown	GCA_016457055.1
Vc514	Pork	2015	Non-O1/O139	China	Unknown	GCA_016457095.1
Vc384	Shrimp	2015	Non-O1/O139	China	Unknown	GCA_016457125.1
Vc383	Pork	2015	Non-O1/O139	China	Unknown	GCA_016457135.1
Vc3012	Shrimp	2019	Non-O1/O139	China	Unknown	GCA_016457155.1
Vc2932	Shrimp	2019	Non-O1/O139	China	Unknown	GCA_016457185.1
Vc3013	Shrimp	2019	Non-O1/O139	China	Unknown	GCA_016457205.1
CISM_0015	Homo sapiens	2002	O1	Mozambique	69	GCA_002099055.1
CISM_0016	Homo sapiens	2002	O1	Mozambique	69	GCA_002099035.1
CISM_0017	Homo sapiens	2002	O1	Mozambique	69	GCA_002099015.1
CISM_0018	Homo sapiens	2002	O1	Mozambique	69	GCA_002098995.1
CISM_0019	Homo sapiens	2002	O1	Mozambique	69	GCA_002098965.1
CISM_100	Homo sapiens	2003	O1	Mozambique	69	GCA_002098835.1
CISM_101	Homo sapiens	2003	O1	Mozambique	69	GCA_002098765.1

CISM_105	Homo sapiens	2003	O1	Mozambique	69	GCA_002098695.1
CISM_120	Homo sapiens	2003	O1	Mozambique	69	GCA_002098675.1
CISM_121	Homo sapiens	2003	O1	Mozambique	69	GCA_002098655.1
CISM_300043	Homo sapiens	2008	O1	Mozambique	69	GCA_002098295.1
CISM_300055	Homo sapiens	2008	Non-O1	Mozambique	766	GCA_002097735.1
CISM_300205	Homo sapiens	2008	Non-O1	Mozambique	733	GCA_002097745.1
CISM_300208	Homo sapiens	2008	O1	Mozambique	69	GCA_002098345.1
CISM_300209	Homo sapiens	2008	O1	Mozambique	69	GCA_002098335.1
CISM_300215	Homo sapiens	2008	O1	Mozambique	69	GCA_002098255.1
CISM_302015	Homo sapiens	2009	O1	Mozambique	69	GCA_002098225.1
CISM_302029	Homo sapiens	2009	O1	Mozambique	69	GCA_002098235.1
BD02	Environmental	2013	Non-O1/O139	Bangladesh	1018	GCA_013111535.1
BD03	Environmental	2013	Non-O1/O139	Bangladesh	1265	GCA_003348515.1
BD04	Environmental	2013	Non-O1/O139	Bangladesh	1274	GCA_003348485.1
BD06	Environmental	2013	Non-O1/O139	Bangladesh	1268	GCA_003348715.1
BD07	Environmental	2013	Non-O1/O139	Bangladesh	1270	GCA_003348675.1
BD10	Environmental	2013	Non-O1/O139	Bangladesh	1277	GCA_003348405.1
BD12	Environmental	2013	Non-O1/O139	Bangladesh	1265	GCA_003348355.1
BD13	Environmental	2013	Non-O1/O139	Bangladesh	1265	GCA_003348395.1
BD14	Environmental	2013	Non-O1/O139	Bangladesh	1106	GCA_003348655.1
BD15	Environmental	2013	Non-O1/O139	Bangladesh	1265	GCA_003348665.1
BD18	Environmental	2013	Non-O1/O139	Bangladesh	1299	GCA_003348365.1
BD20	Environmental	2013	Non-O1/O139	Bangladesh	1265	GCA_003348595.1
7-6-5	<i>Aristichthys nobilis</i>	2018	Non-O1/O139	China	Unknown	SAMN37882014
L10-48	<i>Aristichthys nobilis</i>	2018	Non-O1/O139	China	Unknown	SAMN37882015
L1-1	<i>Ctenopharyngodon idellus</i>	2018	Non-O1/O139	China	884	SAMN37882016
B5-86	<i>Parabramis pekinensis</i>	2018	Non-O1/O139	China	Unknown	SAMN37882017

**Table S3.** The identified repeats in the four *V. cholerae* genomes.

<i>V. cholerae</i> Isolate	Repeat ID	Location	Scaffold Size (bp)	Repeat (bp)		
				Start	End	Length
7-6-5	Scaffold17_TR021	Scaffold17	69,081	156	404	249
	Scaffold24_TR031	Scaffold24	58,709	156	363	208
	Scaffold40_TR046	Scaffold40	32,671	204	376	173
	Scaffold12_TR016	Scaffold12	75,784	257	490	234
L10-48	Scaffold16_TR036	Scaffold16	56,526	156	392	237
	Scaffold8_TR022	Scaffold8	179,095	256	482	227
	Scaffold6_TR020	Scaffold6	301,187	270	478	209
	Scaffold20_TR046	Scaffold20	19,182	298	540	243
L1-1	Scaffold23_TR42	Scaffold23	17,129	1	539	539
	Scaffold23_TR43	Scaffold23	17,129	249	545	297
B5-86	Scaffold7_TR28	Scaffold7	179,132	1	359	359
	Scaffold8_TR31	Scaffold8	148,220	4	582	579
	Scaffold6_TR27	Scaffold6	246,682	110	314	205
	Scaffold11_TR38	Scaffold11	76,558	196	424	229
	Scaffold19_TR50	Scaffold19	11,414	210	469	260

**Table S4.** The identified GIs in the four *V. cholerae* genomes.

<i>V. cholerae</i> Isolate	GI ID	Location	Start (bp)	End (bp)	CDS No	COG No
7-6-5	GI 1	Scaffold2	46,639	60,249	16	15
	GI 2	Scaffold5	16,316	24,037	8	8
	GI 3	Scaffold13	1,507	7,277	7	6
	GI 4	Scaffold14	1,759	7,379	9	0
L10-48	GI 1	Scaffold1	259,476	289,266	23	12
	GI 2	Scaffold2	59,819	68,563	10	4
	GI 3	Scaffold2	606,572	617,062	9	8
	GI 4	Scaffold3	71,330	108,375	36	28
	GI 5	Scaffold5	46,036	70,975	26	14
	GI 6	Scaffold9	28,986	40,521	14	13
	GI 7	Scaffold11	24,988	32,438	10	3
	GI 8	Scaffold15	4,549	5,851	9	3
	GI 9	Scaffold18	27,601	41,655	10	6
	GI 1	Scaffold1	192,650	204,060	14	4
L1-1	GI 2	Scaffold2	278,864	284,922	7	5
	GI 3	Scaffold2	292,972	298,523	7	4
	GI 4	Scaffold3	122,483	129,810	7	7
	GI 5	Scaffold10	76,226	89,255	15	14
	GI 6	Scaffold12	74,102	79,563	8	3
	GI 1	Scaffold2	97,080	123,738	27	18
B5-86	GI 2	Scaffold3	253,804	281,076	32	13
	GI 3	Scaffold5	5,926	18,760	13	11
	GI 4	Scaffold8	57,738	69,124	14	13

**Table S5.** The identified prophages in the four *V. cholerae* genomes.

<i>V. cholerae</i> Isolate	Prophage ID	Location	Prophage Size (bp)	Start (bp)	End (bp)	Predicted Phage	NCBI Accession No.
L10-48	Ph 1	Scaffold2	24,437	40,094	64,530	<i>Burkholderia_cenocepacia</i> _phage_B cepMa	NC_005882
L1-1	Ph 2	Scaffold5	42,272	20,245	62,516	<i>Escherichia</i> _phage_Arg0145	NC_049918
	Ph 1	Scaffold1	38,198	159,833	198,030	<i>Escherichia</i> _phage_lys12581Vzw	NC_049917
	Ph 2	Scaffold2	42,593	278,864	321,456	<i>Vibrio</i> _phage_VHML	NC_004456
	Ph 3	Scaffold7	9,538	45,274	54,811	<i>Vibrio</i> _phage_VCY_phi	NC_016162
B5-86	Ph 1	Scaffold3	40,712	258,721	299,432	Stx2_converting_phage_Stx2a_F45 1	NC_049924

**Table S6:** The identified Ins in the four *V. cholerae* genomes.

<i>V. cholerae</i> Isolate	In ID	Location	Ins Size (bp)	Start (bp)	End (bp)	CDS Gene
7-6-5	In 1	Scaffold13	3,939	80	4,019	3
L10-48	In 1	Scaffold6	4,073	455	4,528	3
L1-1	In 1	Scaffold8	2,759	83	2,842	1
B5-86	In 1	Scaffold5	13,159	53	13,212	7

**Table S7:** The identified ISs in the four *V. cholerae* genomes.

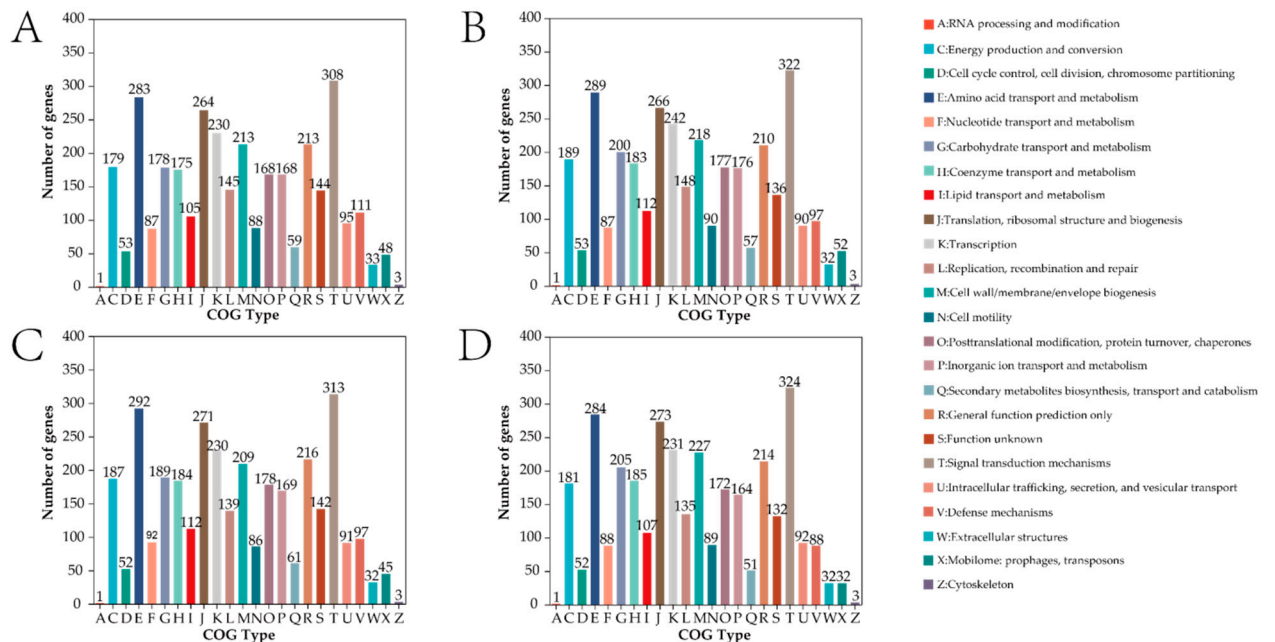
<i>V. cholerae</i> Isolate	IS ID	Location	IS Size (bp)	Start (bp)	End (bp)	IS Family
L10-48	IS001	Scaffold3	1,238	104,563	105,800	ISAs1
	IS003	Scaffold6	1,045	602	1,646	IS30
	IS002	Scaffold3	961	97,205	98,165	IS5
L1-1	IS001	Scaffold50	1,186	1	1,186	IS3
B5-86	IS001	Scaffold9	1,551	59,839	61,389	IS4
	IS002	Scaffold11	620	17,961	18,580	IS200/IS605

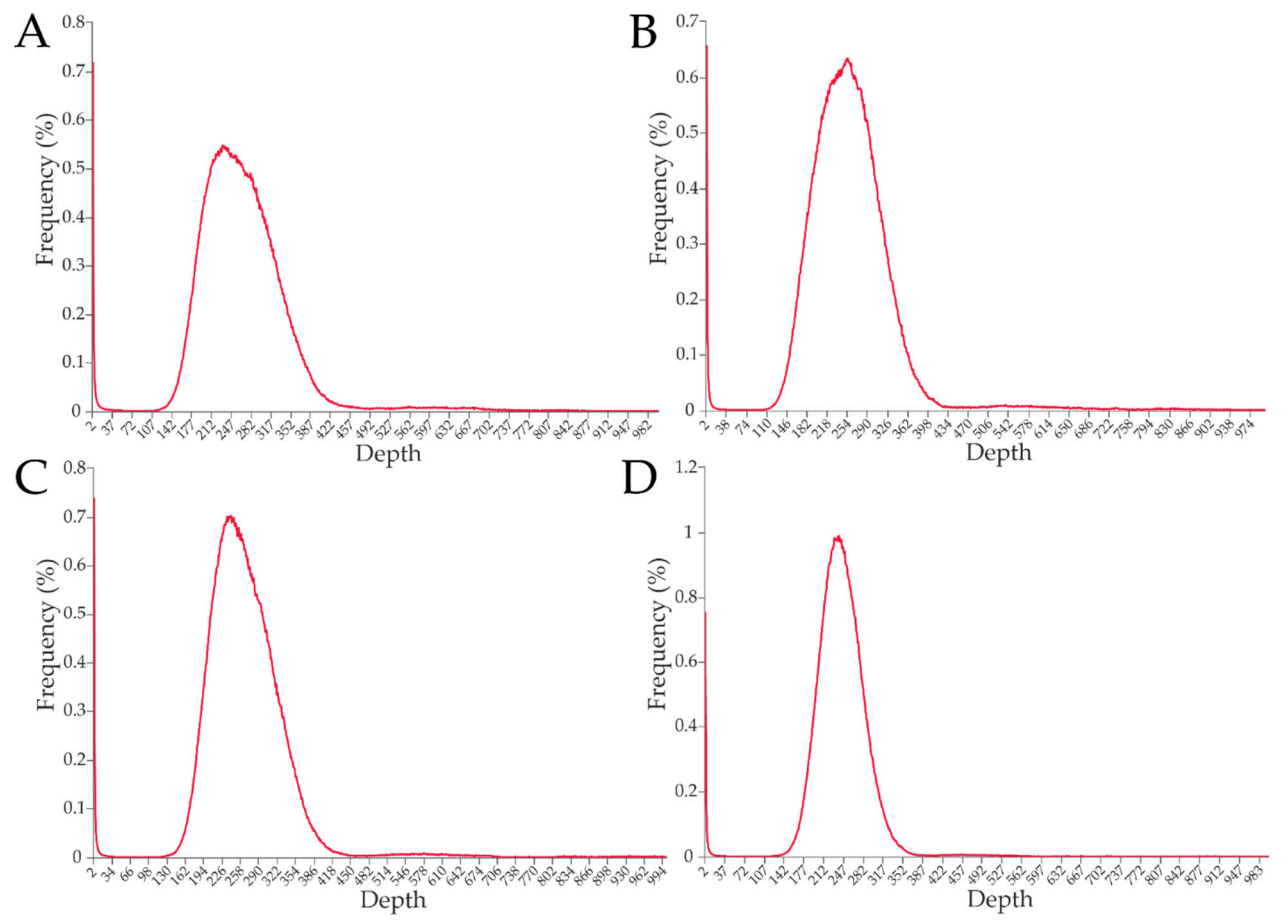
**Table S8:** The identified CRISPR-Cas arrays in the four *V. cholerae* genomes.

<i>V. cholerae</i> Isolate	Location	Cas Type	Start (bp)	End (bp)	Cas Gene
7-6-5	Scaffold3	CAS-Type I F	505,536	506,174	<i>cas6f</i> (Vc 7_6_5_0501)
7-6-5	Scaffold40	CAS-Type I F	2,784,077	2,783,526	<i>cas6f</i> (Vc 7_6_5_2545)
7-6-5	Scaffold64	CAS-Type I F	3,358,954	3,358,316	<i>cas6f</i> (Vc 7_6_5_3049)
L10-48	Scaffold11	CAS-Type I E	3,478,417	3,482,939	<i>casA</i> (Vc L10_48_3096), <i>casB</i> (Vc L10_48_3095), <i>casC</i> (Vc L10_48_3093), <i>casD</i> (Vc L10_48_3092), <i>casE</i> (Vc L10_48_3094)
L1-1	Scaffold2	CAS-Type I F	492,370	491,819	<i>cas6f</i> (Vc L1_1_0413)

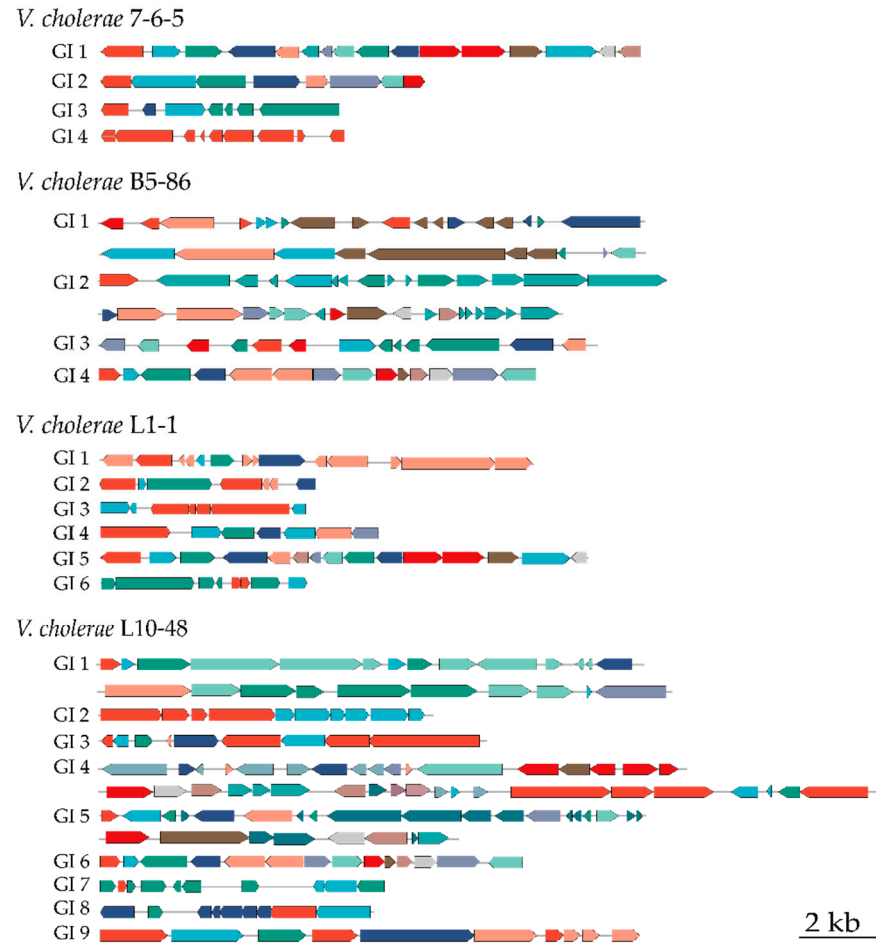
**Table S9.** The potential virulence factors-encoding genes identified in the four *V. cholerae* genomes.

Virulence Factor	<i>V. cholerae</i> Genome	Reference
Flagella	7-6-5, L10-48, L1-1, B5-86	[58]
MSHA pili	7-6-5, L1-1, B5-86	[59]
AI-2	7-6-5, L1-1	[60]
Hcp2	7-6-5, L10-48, L1-1	[61]
LOS	7-6-5, L10-48, L1-1, B5-86	[45]
llpA	7-6-5, L10-48, L1-1, B5-86	[62]
EF-Tu	7-6-5, L10-48, L1-1, B5-86	[44]
RTX toxin	7-6-5, L10-48, L1-1, B5-86	[63]
T6SS	7-6-5, L10-48, L1-1, B5-86	[42]
CAI-1	7-6-5, L10-48, L1-1, B5-86	[64]
T3SS2	L10-48, B5-86	[43]
MARTX	B5-86	[46]
VCC	L1-1, B5-86	[47]
Zot	L1-1, B5-86	[51]

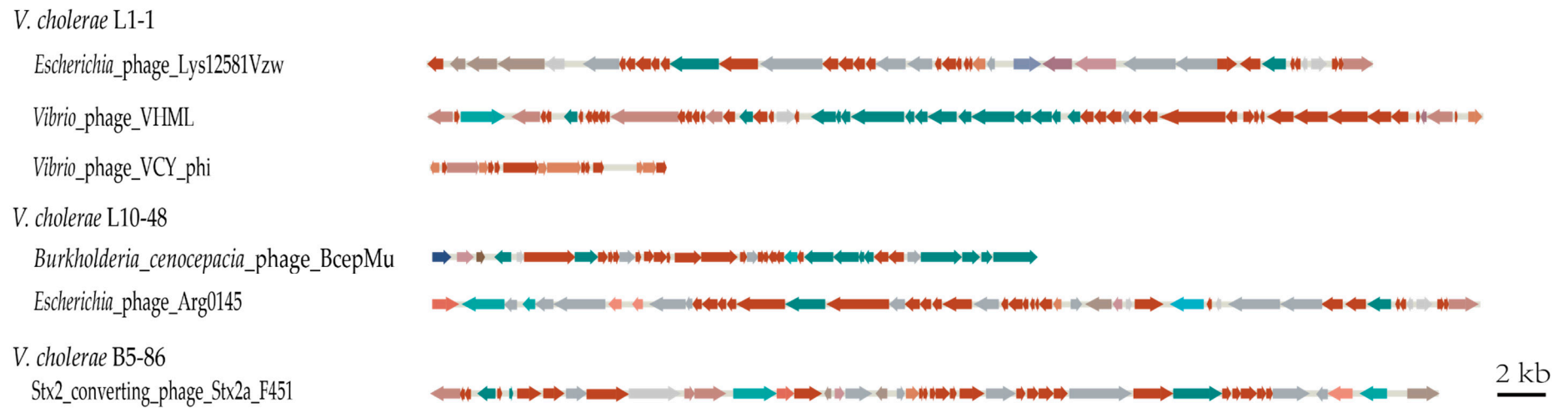
**Figure S1.** The gene function of the GIs identified in the four *V. cholerae* genomes. Different colors referred to COG classification to mark gene function and genes not annotated to COG database were displayed in grey. (A-D), the *V. cholerae* 7-6-5, L10-48, L1-1, and B5-86 genomes, respectively.



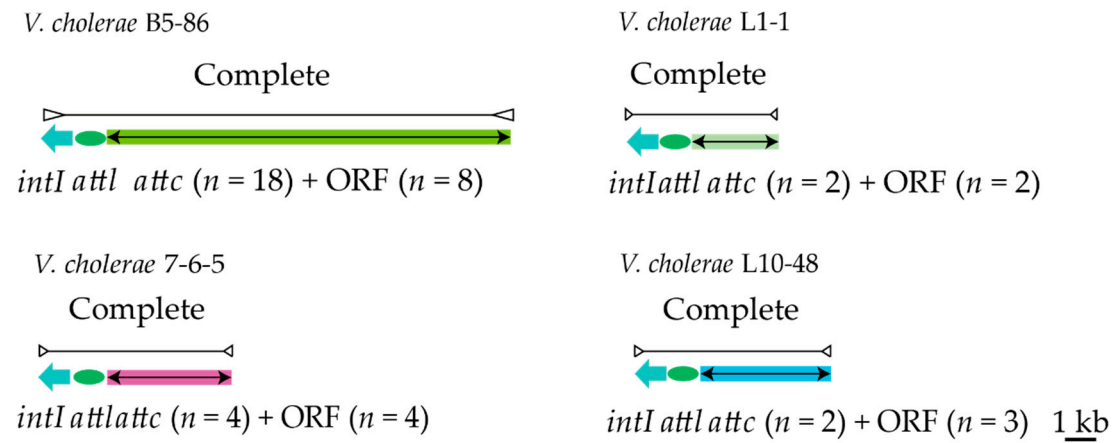
**Figure S2.** The k-mer analysis for the four *V. cholerae* subread data based on the number of unique 17-mers. (A-D), the *V. cholerae* 7-6-5, L10-48, L1-1, and B5-86 genomes, respectively.



**Figure S3.** Gene organizations of the GIs identified in the four *V. cholerae* genomes. Different colors referred to COG classification in Figure S1.



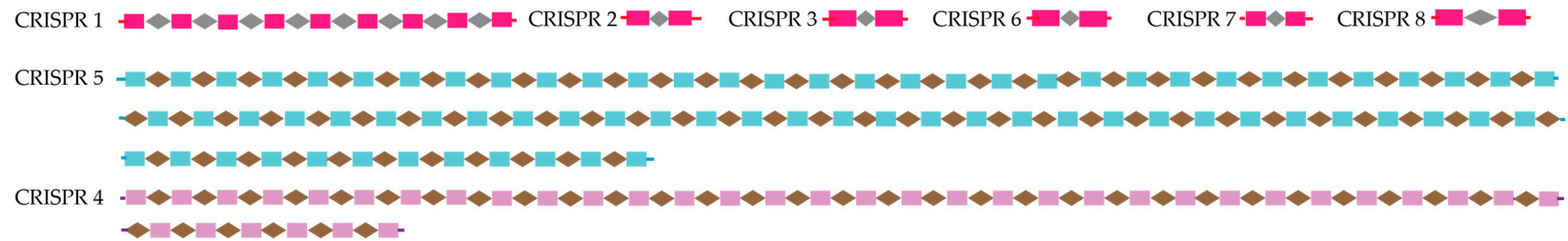
**Figure S4.** The structure diagram of the prophage gene clusters identified in the four *V. cholerae* genomes.



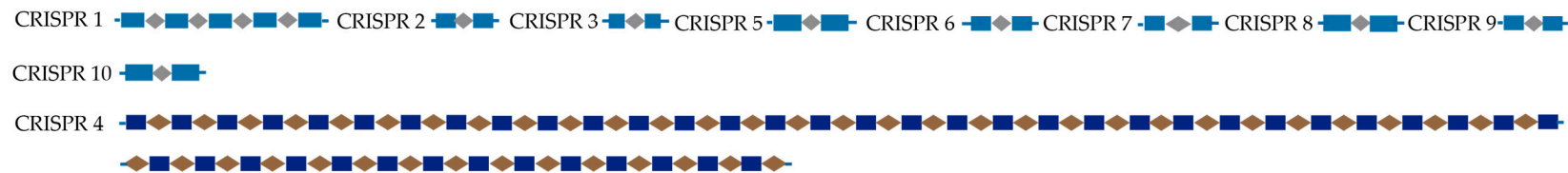
**Figure S5.** The structure diagram of the Ins identified in the four *V. cholerae* genomes.



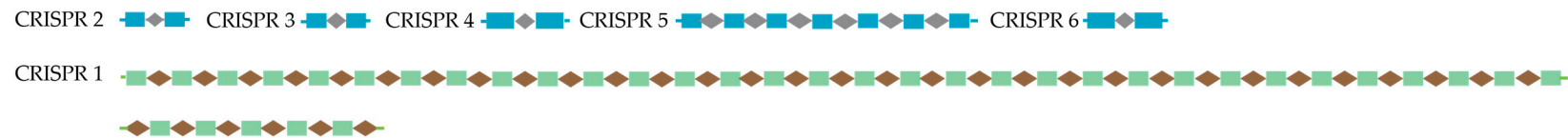
*V. cholerae* 7-6-5



*V. cholerae* L10-48



*V. cholerae* L1-1



*V. cholerae* B5-86



**Figure S6.** The structural features of the CRISPR arrays identified in the four *V. cholerae* genomes. The repeat sequences were shown by the rectangle of different colors and the spacer regions were represented by rhombuses of different colors.

## References

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