

Supplementary information

Genomic characterization of the non-O1/non-O139 *Vibrio cholerae* strain that caused a gastroenteritis outbreak in Santiago, Chile, 2018

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Supplementary Table 1. *Vibrio cholerae* genomes analyzed in this study

Strain ID	Country	Origin / Host	Diagnostic	No. of contigs	GenBank Accession
5473-62	Philippines	Human	Diarrhea	94	JID100000000

1311-69	India	Human	Diarrhea	130	JIDJ00000000
1157-74	India	Human	Diarrhea	151	JIDL00000000
HE-45	Haiti	Environment	-	29	ALED00000000
HC-43B1	Haiti	Human	-	20	ALDP00000000
HC-46B1	Haiti	Human	-	24	AJSL00000000
VcN1	Bangladesh	Environment	-	178	PDNJ00000000
133-73	India	Human	Diarrhea	118	JIDK00000000
984-81	India	Human	Diarrhea	117	JMBM00000000
254-93	India	Human	Diarrhea	114	JMBP00000000
HE-46	Haiti	Environment	-	143	MIPM00000000
HE-48	Haiti	Environment	-	18	AFOR00000000
HC-1A2	Haiti	Human	-	19	AJRO00000000
HC-2A1	Haiti	Human	-	206	AFOT00000000
HC-36A1	Haiti	Human	-	17	AXDR00000000
HC-61A2	Haiti	Human	-	24	AJRU00000000
HC-78A1	Haiti	Human	-	172	AGUZ00000000
HC-50A1	Haiti	Human	-	254	ALDS00000000
HC-51A1	Haiti	Human	-	249	ALDT00000000
HC-52A1	Haiti	Human	-	271	ALDU00000000
HC-55A1	Haiti	Human	-	243	ALDV00000000
HC-56A1	Haiti	Human	-	217	ALDW00000000
HC-57A1	Haiti	Human	-	237	ALDY00000000
G4222	South Africa	Human	Cholera	21	ANNB01000000
NIH41	India	Human	NA	111	JIDH00000000
981-75	India	Human	Diarrhea	152	JIDM00000000
63-93	India	Human	Diarrhea	81	JMBN00000000
A1552	Peru	Human	Cholerae	2	CP025936, CP025937
N16961	Bangladesh	Human	Cholerae	2	NZ_CP028827.1, NZ_CP028828.1
MS6	Thailand	Human	Diarrhea	2	AP014524, AP014525
2012EL-1759	Haiti	Environment	-	68	JNEW00000000
V52	Sudan	Human	-	111	AAKJ00000000
1154-74	India	Human	Diarrhea	1	CP010811
10432-62	Philippines	Human	Diarrhea	1	CP010812
PS15	USA	Environment	-	131	AJJR00000000
8_76	India	Human	Diarrhea	82	JIDN00000000
1421-77	India	Human	Diarrhea	118	JMBL00000000
571-88	China	Human	Diarrhea	117	JIDO00000000
60555434	Australia	Human	Dysuria	95	QKWT00000000
MZO-2	Bangladesh	Human	-	157	MIKJ00000000
TP	USA	Environment	-	291	MIPK00000000
VC53	USA	Oyster	-	247	MIOU00000000
1587	Peru	Human	-	114	AAUR00000000
623-39	Bangladesh	Environment	-	80	AAWG00000000
AM-19226	Bangladesh	Human	-	62	AATY00000000
CT 5369-93	Brazil	Environment	-	269	ADAL00000000
MZO-3	Bangladesh	Human	-	77	AAUU00000000
RC385	USA	Environment	-	97	AAKH00000000
TMA21	Brazil	Environment	-	20	ACHY00000000
HE-25	Haiti	Environment	-	13	ALEC00000000
BJG-01	USA	NA	-	311	AFOU00000000
234-93	India	Human	Diarrhea	129	JMBO00000000
V51	USA	Human	-	143	AAKI00000000
CP1110	USA	Human	Cholera	184	AMWF00000000
CP1111	USA	Human	Cholera	199	AMWS00000000
CP1112	USA	Human	Cholera	193	AMWT00000000
CP1113	USA	Human	Cholera	186	AMWU00000000
CP1114	USA	Human	Cholera	199	AMWV00000000
CP1115	USA	Human	Cholera	183	AMWR00000000
CP1116	USA	Human	Cholera	207	ANNM00000000
CP1117	USA	Human	Cholera	183	AMWW00000000
VC48	USA	Oyster	-	152	MLOT00000000
3568-07	Mexico	Fresh Cheese	-	142	MIPL00000000
VCC19	Brazil	Environment	-	54	ATEV00000000
490-93	Thailand	Human	Diarrhea	274	JIDQ00000000
HE-09	Haiti	Environment	-	258	AFOP00000000
HE-16	Haiti	Environment	-	326	ALEB00000000
SIO	USA	Environment	-	150	MIPJ00000000
S12	Australia	Environment	-	83	MDST01000000

Supplementary Table 2. Virulence associated genes analyzed in this study

Virulence genes	Gene name	GenBank accession	Nucleotide positions
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Filamentous phage CTXΦ			
<i>ctxA</i>	Cholera enterotoxin, A subunit	LT906614.1	c1568126-1567350
<i>ctxB</i>	Cholera enterotoxin, B subunit	LT906614.1	c1567353-1566979
<i>zot</i>	Zonular occludens toxin	LT906614.1	c1569424-1568225
<i>ace_</i>	Accessory cholera enterotoxin	LT906614.1	c1569702-1569409
Vibrio pathogenicity island-I (VPI-I)			
<i>aldA</i>	Aldehyde dehydrogenase	LT906614.1	875278-876903
<i>tcpA</i>	Toxin-coregulated pilin	AF452571.1	
<i>acfC</i>	Accessory colonization factor AcfC	LT906614.1	903385-904155
Vibrio pathogenicity island-II (VPI-II)			
<i>motB-like</i>	Chemotaxis protein MotB-related protein	LT906614.1	c1903654-1902920
<i>nanA</i>	N-acetylneuraminase lyase	LT906614.1	c1927114-1926218
<i>nanK</i>	N-acetylmannosamine kinase	LT906614.1	1930905-1931768
<i>nanH</i>	Neuraminidase	LT906614.1	1933253-1935676
Vibrio Seventh Pandemic Island-I (VSP-I)			
<i>VC0175</i>	Deoxycytidylate deaminase-related protein	LT906614.1	c176941-175343
<i>vspR</i>	<i>V. cholerae</i> seventh pandemic regulator	LT906614.1	177861-178424
<i>VC0178</i>	Patatin-related protein	LT906614.1	179338-180405
<i>dncV</i>	Di-nucleotide cyclase Vibrio	LT906614.1	180419-181729
Vibrio Seventh Pandemic Island-II (VSP-II)			
<i>VC0494</i>	Hypothetical protein	LT906614.1	528305-528949
<i>VC0498</i>	Ribonuclease HI	LT906614.1	530684-531124
<i>VC0502</i>	Type IV pilin	LT906614.1	c534722-534198
<i>VC0512</i>	Methyl-accepting chemotaxis protein	LT906614.1	541318-542907
Other toxins			
<i>hlyA</i>	Vibrio cholerae cytotoxin	LT906615.1	237585-239810
<i>rtxA</i>	MARTX (multifunctional autoprocessing RTX toxin)	AE003852.1	1550108-1563784
<i>chxA</i>	Cholix toxin	AB754476.1	
<i>stn</i>	Heat-stable enterotoxin, NAG-ST	M85198.1	
Other virulence associated genes			
<i>toxR</i>	ToxR, regulator of the expression of virulence factors	LT906614.1	c1047905-1047185
<i>mshA</i>	Mannose-sensitive hemagglutinin (MSHA), a type 4 pilus	LT906614.1	435450-435986
<i>ompU</i>	Outer membrane protein U	KF434513.1	
<i>ompW</i>	Outer membrane protein W	LT906615.1	c820096-819681
Type III secretion system (T3SS)			
<i>vcsN2</i>	Type III secretion cytoplasmic ATPase	CP010812.1	c2624653-2623391
<i>vcsC2</i>	Type II/III secretion system protein	CP010812.1	c2626110-2624653
<i>vcsV2</i>	Type III secretion protein	CP010812.1	c2635653-2633770
<i>vspD</i>	Putative secreted protein D	CP010812.1	c2644010-2642961
<i>tdh2</i>	Thermostable direct hemolysin 2	CP010812.1	2659186-2659728
Type VI secretion system (T6SS)			
<i>vgrG-1</i>	Valine-glycine repeat protein G 1	LT906614.1	1510289-1513780
<i>vgrG-2</i>	Valine-glycine repeat protein G 2	LT906615.1	20426-22510
<i>vgrG-3</i>	Valine-glycine repeat protein G 3	LT906615.1	137579-140632
<i>vgrG-4</i>	Valine-glycine repeat protein G 4	KU722393.1	13902-15878
<i>hcp</i>	Hemolysin coregulated protein	LT906614.1	1509643-1510161

Supplementary Table 3. Open reading frames localized within GIVch-T6SS^{Santiago-089} (GenBank accession: SRLP00000000)

ORF#	Position (bp) *	Denomination in transposable element	Closest informative protein match	Identities	Accession No. of homologue	Function to closest related protein. Comments
	1 - 367	ssrA tmRNA				
1	534 – 1,772	Integrase	Tyrosine-type recombinase/integrase	411/412 (99%)	WP_032480643.1	DNA mobility
	c2,129 – 5,276	CRISPR with 53 repeat units repeat region				
2	c5,432 – 6,070	cas6	Type I-F CRISPR-associated endoribonuclease Cas6/Csy4	211/212 (99%)	WP_142735065.1	CRISPR-Cas system
3	c6,080 – 7,111	csy3	Type I-F CRISPR-associated protein Csy3	343/343 (100%)	WP_142576943.1	CRISPR-Cas system
4	c7,123 – 8,010	csy2	Type I-F CRISPR-associated protein Csy2	295/295 (100%)	WP_142735064.1	CRISPR-Cas system
5	c8,007 – 9,284	csy1	Type I-F CRISPR-associated protein Csy1	425/425 (100%)	WP_142735063.1	CRISPR-Cas system
6	c9,284 – 12,652	cas3	Type I-F CRISPR-associated helicase Cas3	1120/1122 (99%)	WP_071192023.1	CRISPR-Cas system
7	c12,649 – 13,638	cas1	Type I-F CRISPR-associated endonuclease Cas1	327/329 (99%)	WP_119788720.1	CRISPR-Cas system
8	c13,753 – 14,547	Hypothetical protein	WYL domain-containing protein	264/264 (100%)	WP_000880612.1	Predicted DNA-binding transcriptional regulator contains HTH and WYL domains
9	14,963 – 15,481	<i>hcp</i>	Hcp family type VI secretion system effector	172/172 (100%)	WP_001142919.1	Type VI secretion system effector
10	15,608 – 17,584	<i>vgrG-4</i>	VgrG-2 protein	428/620 (69%)	WP_000212125.1	Type VI secretion system effector
11	17,584 – 18,372	Hypothetical protein	DUF4123 domain-containing protein	260/262(99%)	WP_033932339.1	Domain of unknown function (DUF4123)
12	18,362 – 18,670	Hypothetical protein	Hypothetical protein	98/98 (100%)	WP_142735597.1	Unknown

* c: indicates ORFs transcribed on the complementary strand.

Supplementary Table 4. Open reading frames localized within GIVch-MDR^{Santiago-089} (GenBank accession: SRLP00000000)

ORF#	Position (bp) *	Denomination in transposable element	Closest informative protein match	Identities	Accession No. of homologue	Function to closest related protein. Comments
	trmE gene					
1	1 – 1,170	Int-xerC	Site-specific integrase	385/389 (99%)	WP_000703233.1	DNA mobility
2	c1,414 – 1,758	Hypothetical protein	Helix-turn-helix domain-containing protein	114/114 (100%)	WP_063108740.1	Predicted transcriptional regulator
3	c1,818 – 3,488	Hypothetical protein	DUF927 domain-containing protein	555/556 (99%)	WP_095482111.1	Domain of unknown function (DUF927)
4	4,226 – 4,903	Hypothetical protein	Hypothetical protein	224/224 (100%)	WP_032478679.1	Unknown
5	c4,961 – 5,995	Hypothetical protein	Virulence RhuM family protein	337/344 (98%)	WP_023135447.1	Unknown
6	c6,006 – 6,476	Toxin-Antitoxin gene	Type II toxin-antitoxin system death-on-curing family toxin	156/156 (100%)	WP_023135448.1	Toxin-antitoxin system
DR	6,228- 6,242 28,579 – 28,593	AGC - -GCCAGTGAAGAGCG AGCCCGCCAGTGAAGAGCG				
7	c6,478 – 7,491	Hypothetical protein	DNA-binding protein	337/337 (100%)	EBV0095603.1	Unknown
8	c7,484 – 10,729	<i>hsdR</i>	Type I restriction endonuclease subunit R	1081/1081 (100%)	EBV0095602.1	Type I restriction-modification enzymes
9	c10,729 – 12,018	<i>hsdS</i>	Restriction endonuclease subunit S	429/429 (100%)	EBV0095601.1	Type I restriction-modification enzymes
10	c12,015 – 13,997	<i>hsdM</i>	SAM-dependent DNA methyltransferase	660/660(100%)	EBV0095600.1	Type I restriction-modification enzymes
11	c14,061 – 14,558	Toxin-Antitoxin gene	Addiction module antitoxin	165/165 (100%)	EBV0095599.1	Toxin-antitoxin system
12	c14,708 – 15,331	Resolvase	Resolvase	206/207 (99%)	WP_006811352.1	DNA mobility
13	c15,601 – 16,365	IS6100	IS6-like element IS6100 family transposase	254/254 (100%)	WP_077191101.1	DNA mobility
14	c16,900 – 17,394	Hypothetical protein	Hypothetical protein	164/164 (100%)	AFI96177.1	Unknown
15	c17,500 – 18,339	<i>sul1</i>	Sulfonamide-resistant dihydropteroate synthase Sul1	279/279 (100%)	WP_000451390.1	Resistance to sulfonamide
16	c18,333 – 18,680	<i>emrE</i>	Ethidium bromide-methyl viologen resistance protein EmrE	115/115 (100%)	WP_053764310.1	Putative drug-resistant associated gene
17	c18,881 – 19,354	<i>dfrA15</i>	Trimethoprim-resistant dihydrofolate reductase DfrA15	157/157 (100%)	WP_001355915.1	Resistance to trimethoprim
18	19,511 – 20,524	Int-xerD	Class 1 integron integrase IntI1	337/337 (100%)	WP_000845048.1	DNA mobility
19	20,939 – 22,621	<i>tniA</i>	TniA	559/560 (99%)	AJD77018.1	DNA mobility
20	22,624 – 23,532	<i>tniB</i>	AAA family ATPase	302/302 (100%)	WP_000393453.1	DNA mobility
21	23,529 – 24,746	<i>tniQ</i>	TniQ family protein	405/405(100%)	WP_000801210.1	DNA mobility

22	24,807 – 25,421	<i>tniR</i>	Recombinase family protein	204/204 (100%)	WP_000904941.1	DNA mobility
23	c25,474 – 25,710	<i>merE</i>	Broad-spectrum mercury transporter MerE	78/78 (100%)	WP_001087809.1	Mercury resistance
24	c25,707 – 26,072	<i>merD</i>	Mercury resistance co-regulator MerD	121/121 (100%)	WP_003465059.1	Mercury resistance
25	c26,089 – 27,735	<i>merA</i>	Mercury (II) reductase	548/548 (100%)	WP_092230857.1	Mercury resistance
26	c27,732 – 27,977	<i>merF</i>	Mercury resistance system transport protein MerF	81/81 (100%)	WP_000654684.1	Mercury resistance
27	c27,980 – 28,255	<i>merP</i>	Mercury resistance system periplasmic binding protein MerP	91/91 (100%)	WP_000735441.1	Mercury resistance
28	c28,271 – 28,621	<i>merT</i>	Mercuric ion transporter MerT	116/116 (100%)	WP_001294667.1	Mercury resistance
29	c28,693 – 29,145	<i>merR</i>	Hg(II)-responsive transcriptional regulator	144/144 (100%)	WP_000429838.1	Mercury resistance
30	c29,156 – 29,605	Hypothetical protein	Hypothetical protein	146/149 (98%)	WP_001965932.1	Unknown
31	c29,583 – 29,744	Hypothetical protein	Hypothetical protein	53/53 (100%)	EKL05168.1	Unknown
32	c29,731 – 30,162	Hypothetical protein	Hypothetical protein	143/143 (100%)	WP_000834463.1	Unknown
33	c30,159 – 31,001	Hypothetical protein	Hypothetical protein	279/280 (99%)	WP_001971006.1	Unknown

* c: indicates ORFs transcribed on the complementary strand.

Clustal Omega Alignment of the GyrA protein of the Santiago and N16961 strains

GyrA_Santiago	MSDLAKEITPVNIEDELRSYLDYAMSVIVGRALPDVRDGLKPVHRRVLFAMNVLGNDWN	60
GyrA_N16961	MSDLAKEITPVNIEDELRSYLDYAMSVIVGRALPDVRDGLKPVHRRVLFAMNVLGNDWN *****	60
GyrA_Santiago	KAYKKSARVVGDVIGKYHPHGDIAVYDITVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAM	120
GyrA_N16961	KAYKKSARVVGDVIGKYHPHGDIAVYDITVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAM *****	120
GyrA_Santiago	RYTEVRMSKIAHELLADLDKETVDYVYPNYDGTQIPAVLPTKIPNLLINGS;SGIAVGMAT	180
GyrA_N16961	RYTEVRMSKIAHELLADLDKETVDYVYPNYDGTQIPAVLPTKIPNLLINGS;SGIAVGMAT *****	180
GyrA_Santiago	NIPPHNLGEVIDGCLAYIDNEAITIDELMDYIPGPDFPTAALISGRKGIIDAYKTGRGKV	240
GyrA_N16961	NIPPHNLGEVIDGCLAYIDNEAITIDELMDYIPGPDFPTAALISGRKGIIDAYKTGRGKV *****	240
GyrA_Santiago	YMRKAEIETDKNGKETIIVSEIPYQVNKARLIEKIAELVKEKKVEGISALRDESDKDGGM	300
GyrA_N16961	YMRKAEIETDKNGKETIIVSEIPYQVNKARLIEKIAELVKEKKVEGISALRDESDKDGGM *****	300
GyrA_Santiago	RIVIECKRDVAVGEVVLNLYANTQLQTTFGINMVALDNGQPKLFNLKEMLKCFVDHRRREV	360
GyrA_N16961	RIVIECKRDVAVGEVVLNLYANTQLQTTFGINMVALDNGQPKLFNLKEMLKCFVDHRRREV *****	360
GyrA_Santiago	VTRRTIFELRKARERAHILEGLALANIDEIIEELIRRAATPAEAKEGLIARGWDLGNVA	420
GyrA_N16961	VTRRTIFELRKARERAHILEGLALANIDEIIEELIRRAATPAEAKEGLIARGWDLGNVA *****	420
GyrA_Santiago	AMLERAGTDAARPEWLEPQYGIIRDGKYFLTEQQAQAILDLRLHKLTLGLEHEKILDEYKQL	480
GyrA_N16961	AMLERAGTDAARPEWLEPQYGIIRDGKYFLTEQQAQAILDLRLHKLTLGLEHEKILDEYKQL *****	480
GyrA_Santiago	LEEIAALMHILASTERLMEVIREELEAIRAGFSDARRTEITAATHDIDMEELIAREDDVVV	540
GyrA_N16961	LEEIAALMHILASTERLMEVIREELEAIRAGFSDARRTEITAATHDIDMEELIAREDDVVV *****	540
GyrA_Santiago	TLSEHYVKYQLLSDYEAQRGGGKGSATKMKKEEDYIERLLVANTHDNILLFSTRGKTYR	600
GyrA_N16961	TLSEHYVKYQLLSDYEAQRGGGKGSATKMKKEEDYIERLLVANTHDNILLFSTRGKTYR *****	600
GyrA_Santiago	MKVYQLPLASRTARGKPIVNLPLEENERITAILPVTEFSEDKFIFMATGDGTVKKTSLD	660
GyrA_N16961	MKVYQLPLASRTARGKPIVNLPLEENERITAILPVTEFSEDKFIFMATGDGTVKKTSLD *****	660
GyrA_Santiago	QFANVRANGLIALNLRDEDSLIGVDITDGESEIMLFSKFGKVVRFKESEETAVVDENGQP	720
GyrA_N16961	QFANVRANGLIALNLRDEDSLIGVDITDGESEIMLFSKFGKVVRFKESEETAVVDENGQP	720

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GyrA_Santiago      VLDENGGQPEIKFKGVRPMGRTAAGVRGMKLADGDQVVSIVPKTEGDVLTVTENGYGKRT      780
GyrA_N16961       VLDENGGQPEIKFKGVRPMGRTAAGVRGMKLADGDQVVSIVPKTEGDVLTVTENGYGKRT      780
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GyrA_Santiago      SLSEYPTKGRGTQGVVSIKVSERNGSVVGAVQVAEGDEFMMITNAGTLVRRTRVAEVSQVG      840
GyrA_N16961       SLSEYPTKGRGTQGVVSIKVSERNGSVVGAVQVAEGDEFMMITNAGTLVRRTRVAEVSQVG      840
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GyrA_Santiago      RNTQGVTLIRTSEGESVVGLQRIDEIEESELPEGEEALVENDAPVAQDDDDGEQE      894
GyrA_N16961       RNTQGVTLIRTSEGESVVGLQRIDEIEESELPEGEEALVENDAPVAQDDDDGEQE      894
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