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Emergence of *Vibrio cholerae* O1 Sequence Type 75 in Taiwan

Appendix

Experimental Methods

Cholera information and bacterial isolates

We obtained the statistical, demographic, and epidemiologic information on cholera cases in Taiwan from the databases of Taiwan National Infectious Disease Statistics System (https://nidss.cdc.gov.tw/en/) and the National Notifiable Diseases of Surveillance System (*1*) of the Taiwan Centers for Disease Control and *V. cholerae* isolates from the biobank section of Taiwan Centers for Disease Control. A total of 60 isolates were obtained for the study, among which 56 were recovered from cholera patients between 2002 and 2018 and 4 from patients of the cholera outbreak that occurred in 1962 (*2*).

Pulsed-field gel electrophoresis and analysis

We used the PulseNet standardized pulsed-field gel electrophoresis (PFGE) protocol (*3*) to characterize *V. cholerae* isolates, then analyzed PFGE patterns and performed clustering analysis of PFGE patterns using tools provided by BioNumerics 7.6.3 (Applied Maths; http://www.applied-maths.com).

Whole-genome sequencing and sequence analysis

We conducted whole-genome sequencing of *V. cholerae* isolates using Illumina MiSeq sequencing platform (Illumina Inc. USA) with MiSeq Reagent Kit v3 (2X 300 bp), assembled sequence reads using the SPAdes assembler version 3.12.0 (http://cab.spbu.ru/software/spades/), identified sequence types (STs) using the *Vibrio cholerae* core genome multilocus sequence typing MLST database (https://pubmlst.org/v*cholerae*) and antimicrobial resistance genes using the ResFinder tool of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).

Construction of a whole genome single nucleotide polymorphism tree for *V. cholerae* strains from Taiwan

We used BioNumerics version 7.6.3 to construct a minimal spanning tree with whole genome single nucleotide polymorphism (wgSNP) profiles of *V. cholerae* strains. The sequences of raw reads were mapped to the reference genomic sequence of *V. cholerae* strain N16961 (GenBank accession no. GCA_000006745.1) and the mapped sequences of strains and the reference were aligned for SNP calling by using the option of strict SNP filtering (closed SNP set). By using this SNP calling criteria, SNPs are called by removing positions with at least one ambiguous base (non-ATGC base), one unreliable base (N), one gap and non-informative SNPs. Each retained SNP position has minimum 5x coverage, at least covered once in both forward and reverse direction. The minimum distance between retained SNP positions is 12 bp. A dendrogram was constructed with the whole genome SNP profiles using the categorical (SNPs) option for similarity coefficient and minimum spanning tree algorithm for cluster analysis.

Construction of a core genome multilocus sequence typing tree for *V. cholerae* strains from Taiwan and NCBI database

We downloaded genomic sequences from the SRA and Assembly databases of the National Center for Biotechnology Information (NCBI), assembled raw reads using the SPAdes assembler version 3.12.0, and generated core genome multilocus sequence typing (cgMLST) profiles (based on 2,951 core genes) using the in-house developed cgMLST Profiling tool in the cgMLST@Taiwan Web service platform (https://rdvd.cdc.gov.tw/cgMLST) (unpublished). We compared the cgMLST profiles of 60 isolates from Taiwan with those (5,048) from the NCBI database and selected the most match profiles with the Taiwanese isolates to construct a cgMLST genetic relatedness tree using the unweighted pair-group method with arithmetic means algorithm.

References

1. Jian SW, Chen CM, Lee CY, Liu DP. Real-time surveillance of infectious diseases: Taiwan's experience. Health Secur. 2017;15:144–53.

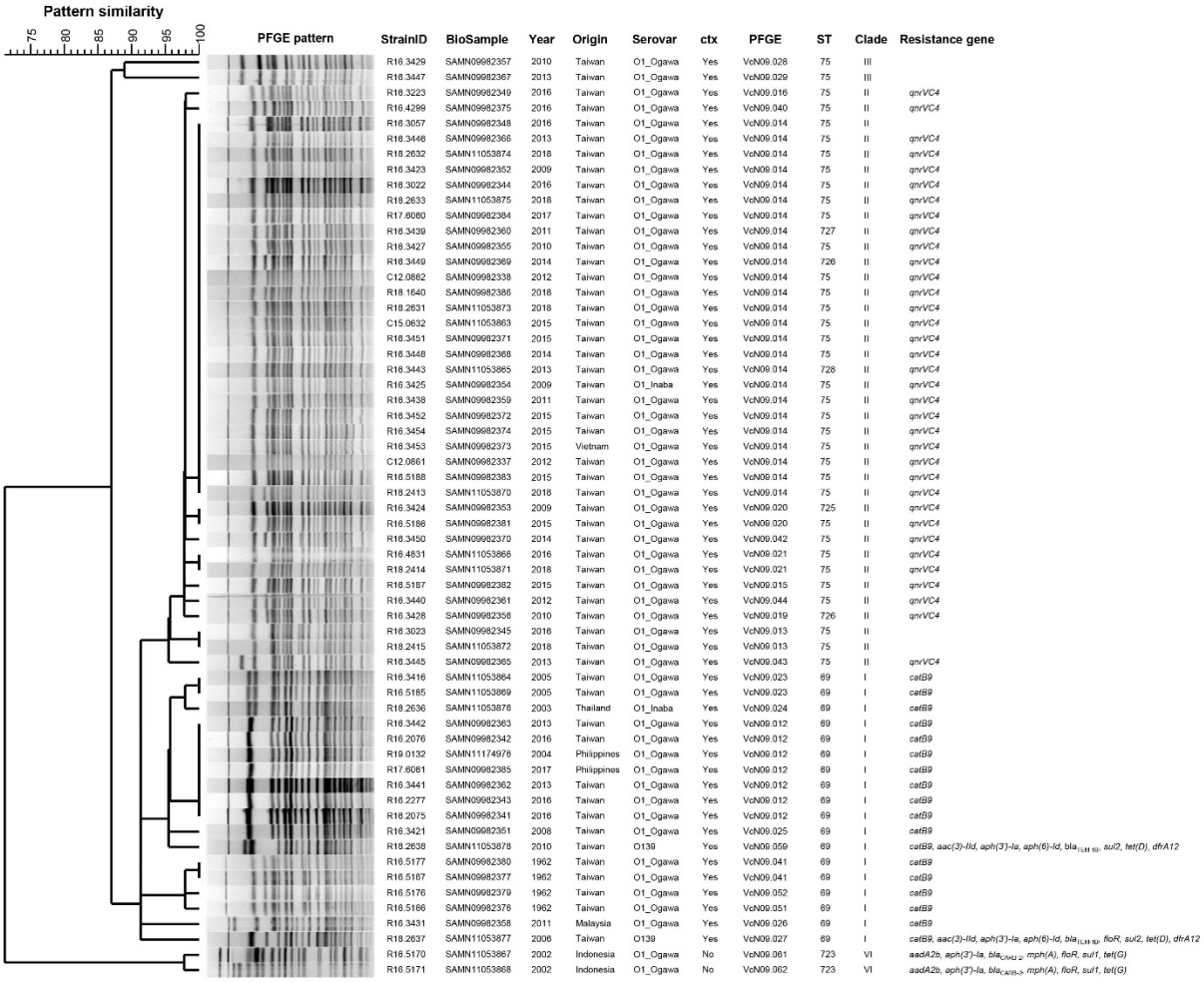
2. Yen CH. A recent study of cholera with reference to an outbreak in Taiwan in 1962. Bull World Health Organ. 1964;30:811–25.

3. Cooper KL, Luey CK, Bird M, Terajima J, Nair GB, Kam KM, et al. Development and validation of a PulseNet standardized pulsed-field gel electrophoresis protocol for subtyping of *Vibrio cholerae.* Foodborne Pathog Dis. 2006;3:51–8.

Appendix Table. Details of *Vibrio cholerae* strains from cholera cases in Taiwan, 2002–2018

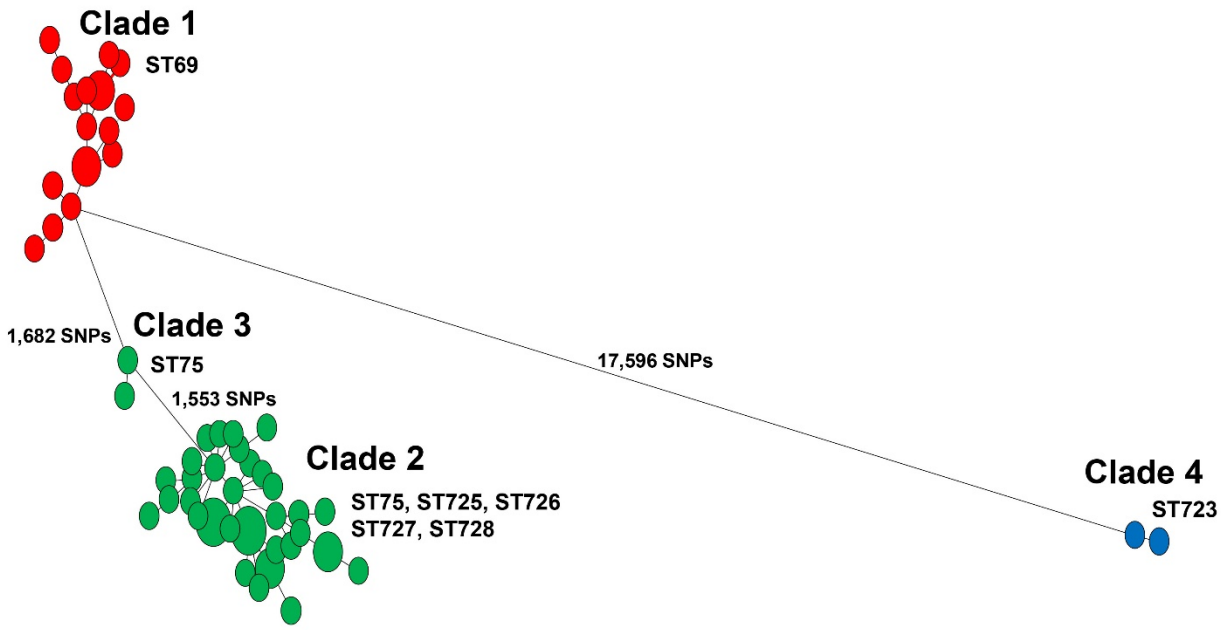
Clade	Sequence type	Variants	No. of isolates	Resistance genes	Country of origin
1	ST69		18	*catB9*§	Taiwan, Thailand, Philippines, Malaysia
2	ST75	ST725*, ST726*, ST728*, ST727†	38	*qnrVC4*¶ (in 35 of 38 isolates)	Taiwan, Vietnam
3	ST75		2	None	Taiwan
4	ST723		2‡	None	Indonesia

*Single-locus variant
†Double-locus variant
‡Nontoxigenic
§Resistant to chloramphenicol
¶Resistant to quinolone

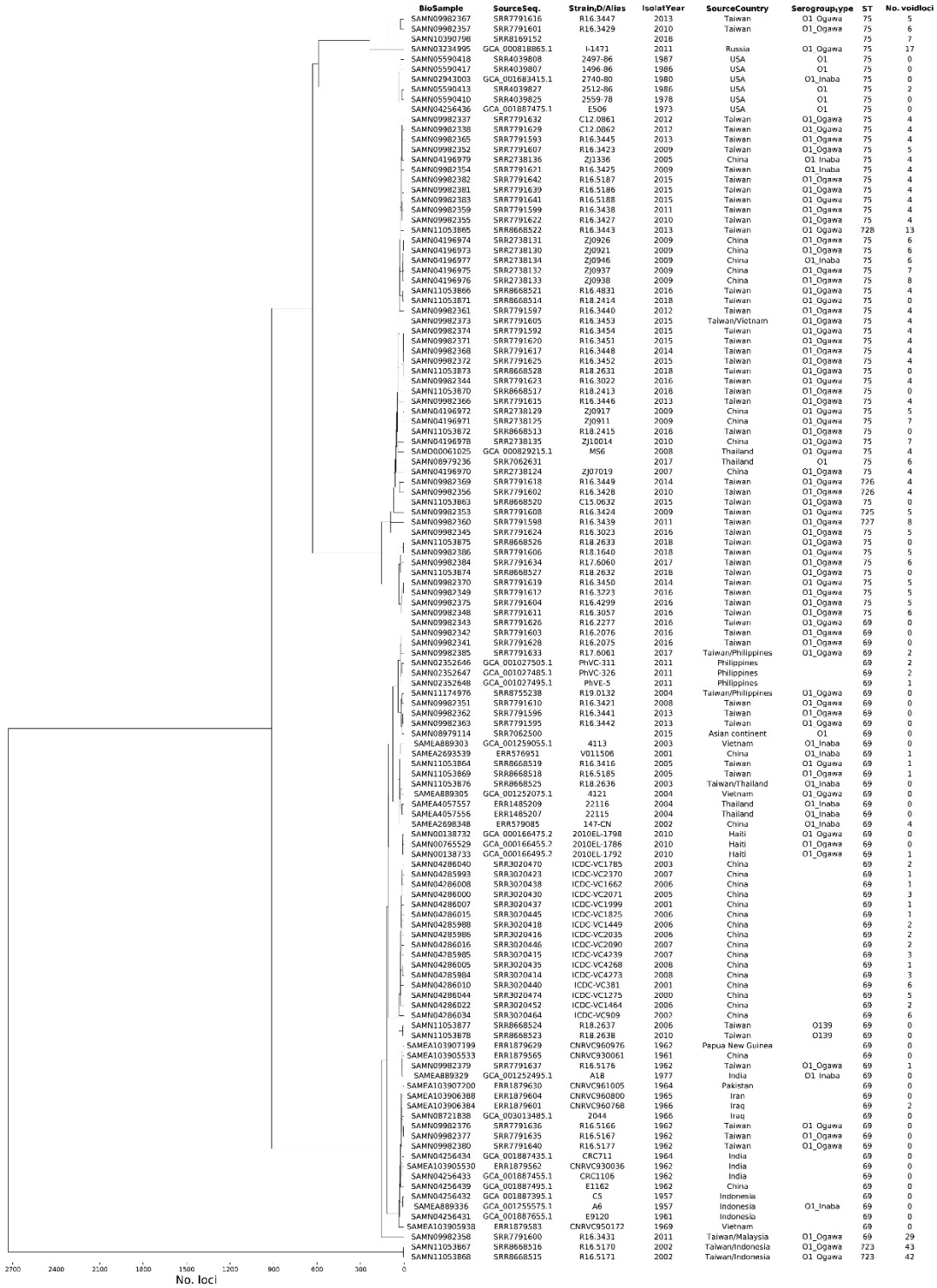


PFGE pattern	StrainID	BioSample	Year	Origin	Serovar	ctx	PFGE	ST	Clade	Resistance gene
	R16.3429	SAMN09982357	2010	Taiwan	O1_Ogawa	Yes	VcN09.028	75	III	
	R18.3447	SAMN09982367	2013	Taiwan	O1_Ogawa	Yes	VcN09.029	75	III	
	R18.3223	SAMN09982349	2016	Taiwan	O1_Ogawa	Yes	VcN09.016	75	II	*qnrVC4*
	R16.4299	SAMN09982375	2016	Taiwan	O1_Ogawa	Yes	VcN09.040	75	II	*qnrVC4*
	R16.3057	SAMN09982348	2016	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	
	R18.3446	SAMN09982366	2013	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.2632	SAMN11053874	2018	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3423	SAMN09982352	2009	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.3022	SAMN09982344	2016	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.2633	SAMN11053875	2018	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R17.6080	SAMN09982384	2017	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.3439	SAMN09982360	2011	Taiwan	O1_Ogawa	Yes	VcN09.014	727	II	*qnrVC4*
	R18.3427	SAMN09982355	2010	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.3449	SAMN09982369	2014	Taiwan	O1_Ogawa	Yes	VcN09.014	726	II	*qnrVC4*
	C12.0862	SAMN09982338	2012	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.1640	SAMN09982386	2018	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.2631	SAMN11053873	2018	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	C15.0632	SAMN11053863	2015	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3451	SAMN09982371	2015	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3448	SAMN09982368	2014	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3443	SAMN11053865	2013	Taiwan	O1_Ogawa	Yes	VcN09.014	728	II	*qnrVC4*
	R16.3425	SAMN09982354	2009	Taiwan	O1_Inaba	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3438	SAMN09982359	2011	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3452	SAMN09982372	2015	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3454	SAMN09982374	2015	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3453	SAMN09982373	2015	Vietnam	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	C12.0861	SAMN09982337	2012	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.5188	SAMN09982383	2015	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R18.2413	SAMN11053870	2018	Taiwan	O1_Ogawa	Yes	VcN09.014	75	II	*qnrVC4*
	R16.3424	SAMN09982353	2009	Taiwan	O1_Ogawa	Yes	VcN09.020	725	II	*qnrVC4*
	R16.5186	SAMN09982381	2015	Taiwan	O1_Ogawa	Yes	VcN09.020	75	II	*qnrVC4*
	R16.3450	SAMN09982370	2014	Taiwan	O1_Ogawa	Yes	VcN09.042	75	II	*qnrVC4*
	R16.4831	SAMN11053866	2016	Taiwan	O1_Ogawa	Yes	VcN09.021	75	II	*qnrVC4*
	R18.2414	SAMN11053871	2018	Taiwan	O1_Ogawa	Yes	VcN09.021	75	II	*qnrVC4*
	R16.5187	SAMN09982382	2015	Taiwan	O1_Ogawa	Yes	VcN09.015	75	II	*qnrVC4*
	R16.3440	SAMN09982361	2012	Taiwan	O1_Ogawa	Yes	VcN09.044	75	II	*qnrVC4*
	R16.3428	SAMN09982356	2010	Taiwan	O1_Ogawa	Yes	VcN09.019	726	II	*qnrVC4*
	R18.3023	SAMN09982345	2016	Taiwan	O1_Ogawa	Yes	VcN09.013	75	II	
	R18.2415	SAMN11053872	2018	Taiwan	O1_Ogawa	Yes	VcN09.013	75	II	
	R18.3445	SAMN09982365	2013	Taiwan	O1_Ogawa	Yes	VcN09.043	75	II	*qnrVC4*
	R16.3416	SAMN11053864	2005	Taiwan	O1_Ogawa	Yes	VcN09.023	69	I	*catB9*
	R16.5185	SAMN11053869	2005	Taiwan	O1_Ogawa	Yes	VcN09.023	69	I	*catB9*
	R18.2636	SAMN11053876	2003	Thailand	O1_Inaba	Yes	VcN09.024	69	I	*catB9*
	R18.3442	SAMN09982363	2013	Taiwan	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R16.2076	SAMN09982342	2016	Taiwan	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R19.0132	SAMN11174976	2004	Philippines	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R17.6081	SAMN09982385	2017	Philippines	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R16.3441	SAMN09982362	2013	Taiwan	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R16.2277	SAMN09982343	2016	Taiwan	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R18.2075	SAMN09982341	2016	Taiwan	O1_Ogawa	Yes	VcN09.012	69	I	*catB9*
	R16.3421	SAMN09982351	2008	Taiwan	O1_Ogawa	Yes	VcN09.025	69	I	*catB9*
	R18.2638	SAMN11053878	2010	Taiwan	O139	Yes	VcN09.059	69	I	*catB9, aac(3)-IId, aph(3')-Ia, aph(6)-Id,* bla_{CMY-9}*, sul2, tet(D), dfrA12*
	R16.5177	SAMN09982380	1962	Taiwan	O1_Ogawa	Yes	VcN09.041	69	I	*catB9*
	R16.5167	SAMN09982377	1962	Taiwan	O1_Ogawa	Yes	VcN09.041	69	I	*catB9*
	R16.5176	SAMN09982379	1962	Taiwan	O1_Ogawa	Yes	VcN09.052	69	I	*catB9*
	R16.5166	SAMN09982378	1962	Taiwan	O1_Ogawa	Yes	VcN09.051	69	I	*catB9*
	R16.3431	SAMN09982358	2011	Malaysia	O1_Ogawa	Yes	VcN09.026	69	I	*catB9*
	R18.2637	SAMN11053877	2006	Taiwan	O139	Yes	VcN09.027	69	I	*catB9, aac(3)-IId, aph(3')-Ia,* bla_{CMY-9}*, aph(6)-Id,* bla_{CMY-9}*, floR, sul2, tet(D), dfrA12*
	R16.5170	SAMN11053867	2002	Indonesia	O1_Ogawa	No	VcN09.061	723	VI	*aadA2b, aph(3')-Ia,* bla_{CARB-2}*, mph(A), floR, sul1, tet(G)*
	R16.5171	SAMN11053868	2002	Indonesia	O1_Ogawa	No	VcN09.062	723	VI	*aadA2b, aph(3')-Ia,* bla_{CARB-2}*, mph(A), floR, sul1, tet(G)*

Appendix Figure 1. Genetic relationships among 60 Vibrio cholerae isolates, with corresponding information. The dendrogram was constructed using PFGE patterns and the single linkage algorithm provided in BioNumerics software version 7.6.3, with settings of 1.5% optimization and 0.95% tolerance.



Appendix Figure 2. A minimum spanning tree for 60 Vibrio cholerae isolates from Taiwan collected in 1962 and in 2002–2018. The tree was constructed with whole genome single-nucleotide polymorphism profiles comprising 20,639 SNPs.



Appendix Figure 3. A genetic dendrogram for 60 Vibrio cholerae isolates collected in Taiwan and their most closely related strains in the NCBI database. Three strains from the 2010 Haiti cholera outbreak were included for comparison. The tree was constructed with cgMLST profiles using the unweighted pair-group method with arithmetic means algorithm.