Supplementary Materials

Table S1: List of primers for virulence and regulatory genes, Vibrio seventh pathogenicity island and PCR conditions used in this study.

Primer	Target	Sequence	Size in bp	PCR Cyclic conditions	References
toxR-F	*toxR*	CCTTCGATCCCCTAAGCAATAC	779	ID: 94°C for 2 min D: 94°C for 1 min A: 62°C for 1 min (20 cycles) E: 72 °C for 1 min D: 94°C for 1 min A: 54°C for 1 min (10 cycles) E: 72 °C for 1 min FE:72°C for 10 min	Singh et al. 2002
toxR-R		AGGGTTAGCAACGATGCGTAAG			
ace-F	*ace*	TAAGGATGTGCTTATGATGGACACCC	316		
ace-R		CGTGATGAATAAAGATACTCATAGG			
tcpA-F		CACGATAAGAAAACCGGTCAAGAG			
tcpA-class	$tcpA^{class}$	TTACCAAATGCAACGCCGAATG	620		
tcpA-El Tor	$tcpA^{ET}$	CGAAAGCACCTTCTTTCACACGTTG	453		
ctxA-F	*ctxA*	CGGGCAGATTCTAGACCTCCTG	564		
ctxA-R		CGATGATCTTGGAGCATTCCCAC			
ompU-F	*ompU*	ACGCTGACGGAATCAACCAAAG	869		
ompU-R		GCGGAAGTTTGGCTTGAAGTAG			
zot-F	*zot*	TCGCTTAACGATGGCGCGTTTT	947		
zot-R		AACCCCGTTTCACTTCTACCCA			
tcpI-F	*tcpI*	TAGCCTTAGTTCTCAGCAGGCA	862	D: 94°C for 1 min A: 62°C for 1 min (35 cycles) E: 72 °C for 1 min	Faruque et al. 1998
tcpI-R		GGCAATAGTGTCGAGCTCGTTA			
hylA ^{ET}F	$hylA^{class/El\ Tor}$	GGCAAACAGCGAAACAAATACC	481	ID: 94°C for 2 min D: 94°C for 1 min A: 62°C for 1 min (30 cycles) E: 72 °C for 1 min EF:72°C for 10 min	Rivera et al. 2001
hylA ^{Cl}F		GAGCCGGCATTCATCTGAAT	727		
hylA-R		CTCAGCGGGCTAATACGGTTTA			

ctxB-F	*ctxB*	GATACACATAATAGAATTAAGGATG	449	ID: 94°C for 2 min D: 94°C for 1 min A: 57°C for 30 sec E: 72 ˚C for 30 sec FE:72°C for 10 min	Mohapatra et al. 2011
ctxB-R		GGTTGCTTCTCATCATCGAACCCAC			
rstR^{class}-F	*rstR*^{class}	TCTCATCAGCAAAGCCTCCATC	243	ID: 94°C for 2 min D: 94°C for 30 sec A: 62°C for 30 sec E: 72 ˚C for 30 sec FE:72°C for 10 min	
rstR^{class}-R		GTAGCAAATGGTATCGGCGTTGG			
rstR^{ET}-F	*rstR*$^{El\ Tor}$	GCACCATGATTTAAGATGCTC	320		
rstR^{ET}-R		GGCAATTAATAAGACTCAGGCAC			
CII-F	chromosomal location of CTXphage	CTCACGCTGAACAGCAAGTC	800	ID: 94°C for 2 min D: 94°C for 1 min A: 52°C for 1 min E: 72 ˚C for 1 min FE:72°C for 10min	Maiti et al. 2006.
CII-R		TTGCTTGAATCGAAAGGACA			
ctxB-3	*ctxB*^{Haiti}	GTTTTACTATCTTCAGCATATGCGA	191	ID: 94°C for 2 min D: 94°C for 30 sec A: 56°C for 30 sec E: 72 ˚C for 30 sec FE:72°C for 10 min	Naha et al. 2012 (DMAMA-PCR)
Rv-cla		CCTGGTACTTCTACTTGAAACG			
ctxB-4	*ctxB*^{class}	GTTTTACTATCTTCAGCATATGCGC	191	ID: 94°C for 2 min D: 94°C for 30 sec A: 60°C for 30 sec E: 72 ˚C for 30 sec FE:72°C for 10 min	
Rv-cla		CCTGGTACTTCTACTTGAAACG			

Fw-com	*ctxB*$^{El\ Tor}$	ACTATCTTCAGCATATGCACATGG	254	ID: 94°C for 2 min D: 94°C for 30 sec A: 54°C for 30 sec } 30 cycles E: 72 ˚C for 30 sec FE:72°C for 10 min	Morita et al.2008 (MAMA-PCR)
Re-elt		CCTGGTACTTCTACTTGAAACA			
smp-F-VC2346	*smp*	GCAACTGTGCTAGCAGTTGCCGTG	405	ID: 94°C for 2 min D: 94°C for 1 min A: 65°C for 30 sec } 30 cycles E: 72 ˚C for 30 sec FE:72°C for 10 min	Grim et al.2010
smp-R-VC2346		GCCTGTTTCAAACGTGATGCGTA			
dcd-820F	*VC0175*	GCTTATTCAGC GCCCTCAGG TC	584	ID: 94°C for 2 min D: 94°C for 1 min A: 65°C for 30 sec } 30 cycles E: 72 ˚C for 30 sec FE:72°C for 10 min	
dcd-1403R		TAG CGT CGA GAT GAC ACA CCT TCG			
VSPI-F	VC0180-VC0181	GCCGAGAACTCTAAAGCG CTTCTC	331	ID: 94°C for 2 min D: 94°C for 1 min A: 61°C for 30 sec } 30 cycles E: 72 ˚C for 30 sec FE:72°C for 10 min	
VSPI-R		CCAAGGTACAGATGAGTACCAGCA			
VC0174-F	Chr-I –VSPI insertion	AAACTGGCGACCTTTGAGCAAGC	1321	ID: 94°C for 2 min D: 94°C for 1 min A: 65°C for 1 min } 30 cycles E: 72 ˚C for 1 min FE:72°C for 10 min	
VC0186-R		GATGGTAGCCTGACGCTGCATCTG			

VCA0695-F	Chr-II-VSPII insertion	ATAGCGGGAGTTGGCTCTGCA	957	ID: 94°C for 2 min	
VCA0697-R		GGTGACTTGGTGCCCATCGTA		D: 94°C for 1 min A: 65°C for 1 min E: 72 ˚C for 1 min 30 cycles FE:72°C for 10 min	

ID: Initial denaturation; D: Denaturation; A: Annealing; E: Extension; FE: Final extension.

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Table S2. Multilocus sequence typing (MLST) primers for five housekeeping genes and PCR conditions used in this study.

Gene	Gene Product	Direction	Primer Sequence(s)	PCR cyclic conditions	References
dnaE	DNA polymerase III alpha subunit	Forward	CgRATMACCgCTTTCgCCg	ID: 98˚C for 30 sec D: 98˚C for 10 sec A: 50-52˚C for 30 sec } 35 cycles E: 70˚C for 30 sec FE:70˚C for 10 min	Garg et al. 2003
		Reverse	gAKATgTgTgAgCTgTTTgC		
lap	Leucine amino-peptidase	Forward	gAAgAggTCggTTTgCgAgg		
		Reverse	gTTTgAATggTgAgCggTTTgCT		
pgm	Phosphoglucomutase	Forward	CCKTCSCAYAACCCgCC		
		Reverse	TCRACRAACCATTTgAADCC		
recA	Recombination Repair Protein	Forward	gAAACCATTTCgACCggTTC		Kotetishvili et al. 2003
		Reverse	CCgTTATAgCTgTACCAAgCgCCC		
asd	Aspartate semialdehyde dehydrogenase	Forward	CgACTACgACATTCCTC		Karaolis et al. 1995
		Reverse	gTTATCCgCCCACTACCC		

ID: initial denaturation; D: denaturation; A: Annealing; E: extension; FE: final extension.

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Table S3. Temporal effect of DNase I, Proteinase K, and NaIO4on biofilm grown in Leuria Bertani Broth (Mann-Whitney test).

Sr. No	Treatment of Bio-film with different reagents	Mann-Whitney test (*p-value <0.05*)
1	Control Vs DNase I	< 0.0001
2	Control Vs Proteinase K	0.0043
3	Control Vs $NaIO_4$	<0.0001

Fig. S1a. Polymorphic sites of *asd* loci among *V. cholerae* isolates. The nucleotide designation was shown for allele one and for rest of alleles sites only polymorphism is shown along with synonymous and non-synonymous polymorphism.

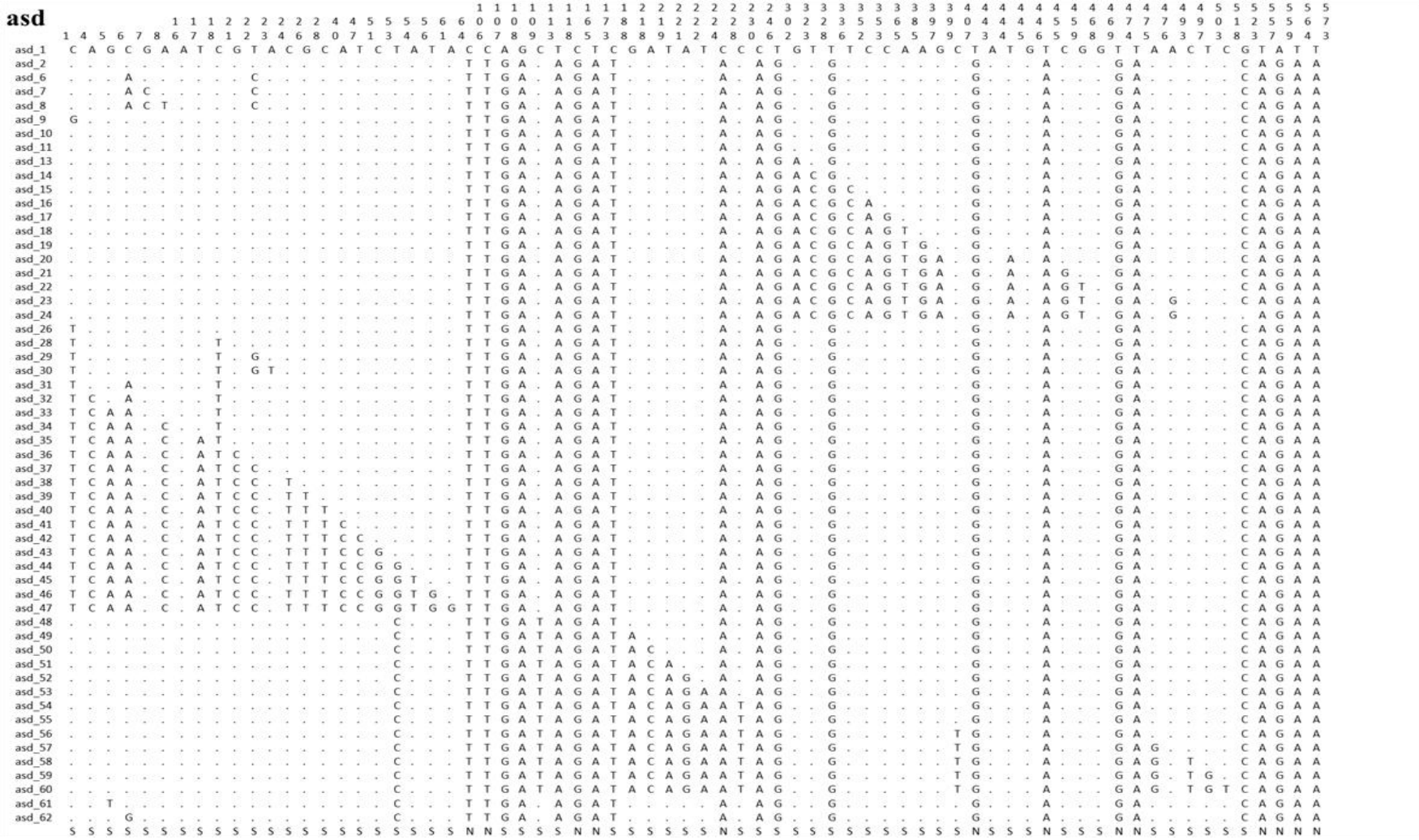


Fig. S1b. Polymorphic sites of *dnaE* loci among *V. cholerae* isolates. The nucleotide designation was shown for allele one and for rest of alleles sites only polymorphism is shown along with synonymous and non-synonymous polymorphism.

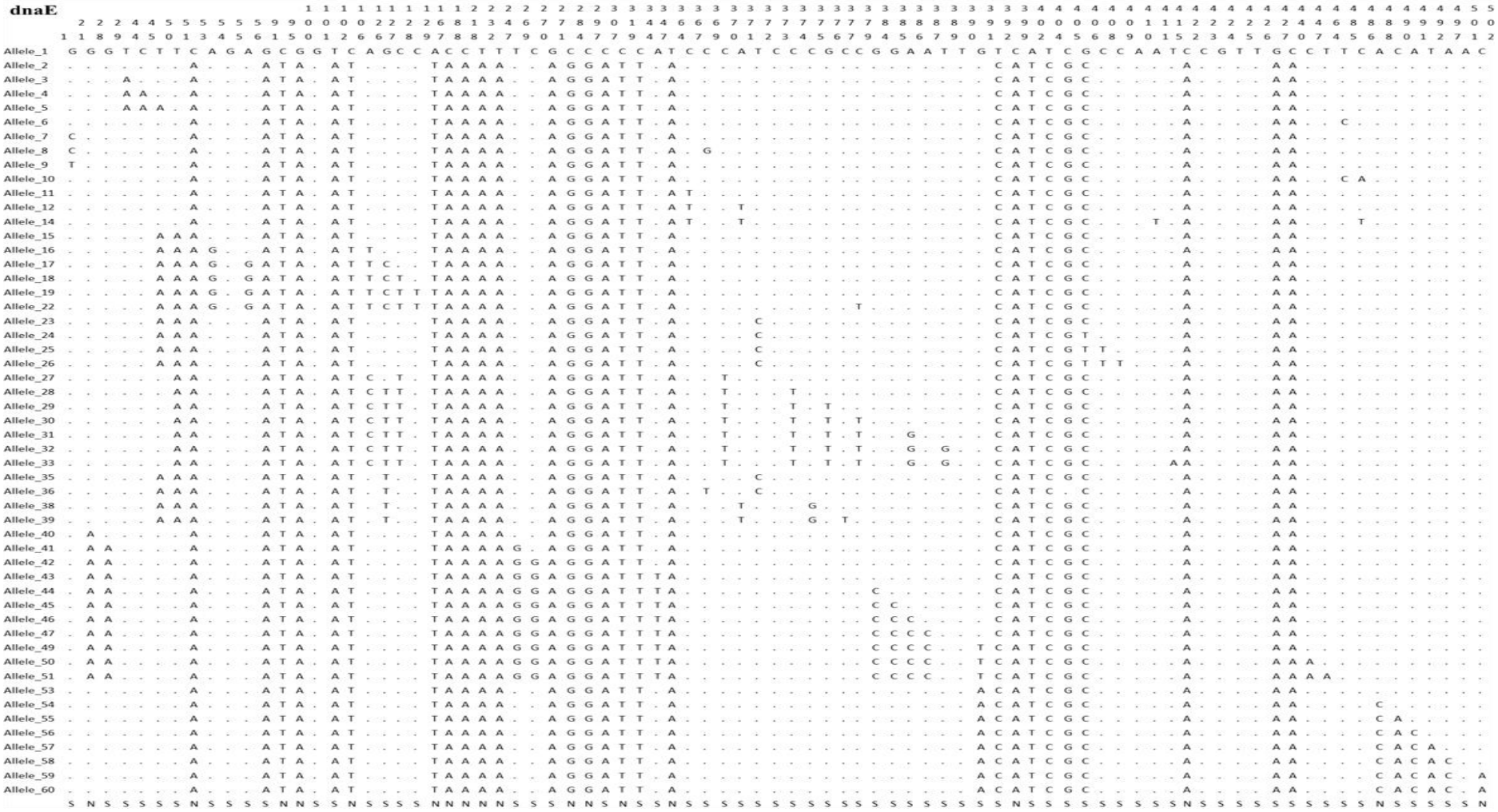


Fig. S1c. Polymorphic sites of *lap* loci among *V. cholerae* isolates. The nucleotide designation was shown for allele one and for rest of alleles sites only polymorphism is shown along with synonymous and non-synonymous polymorphism.

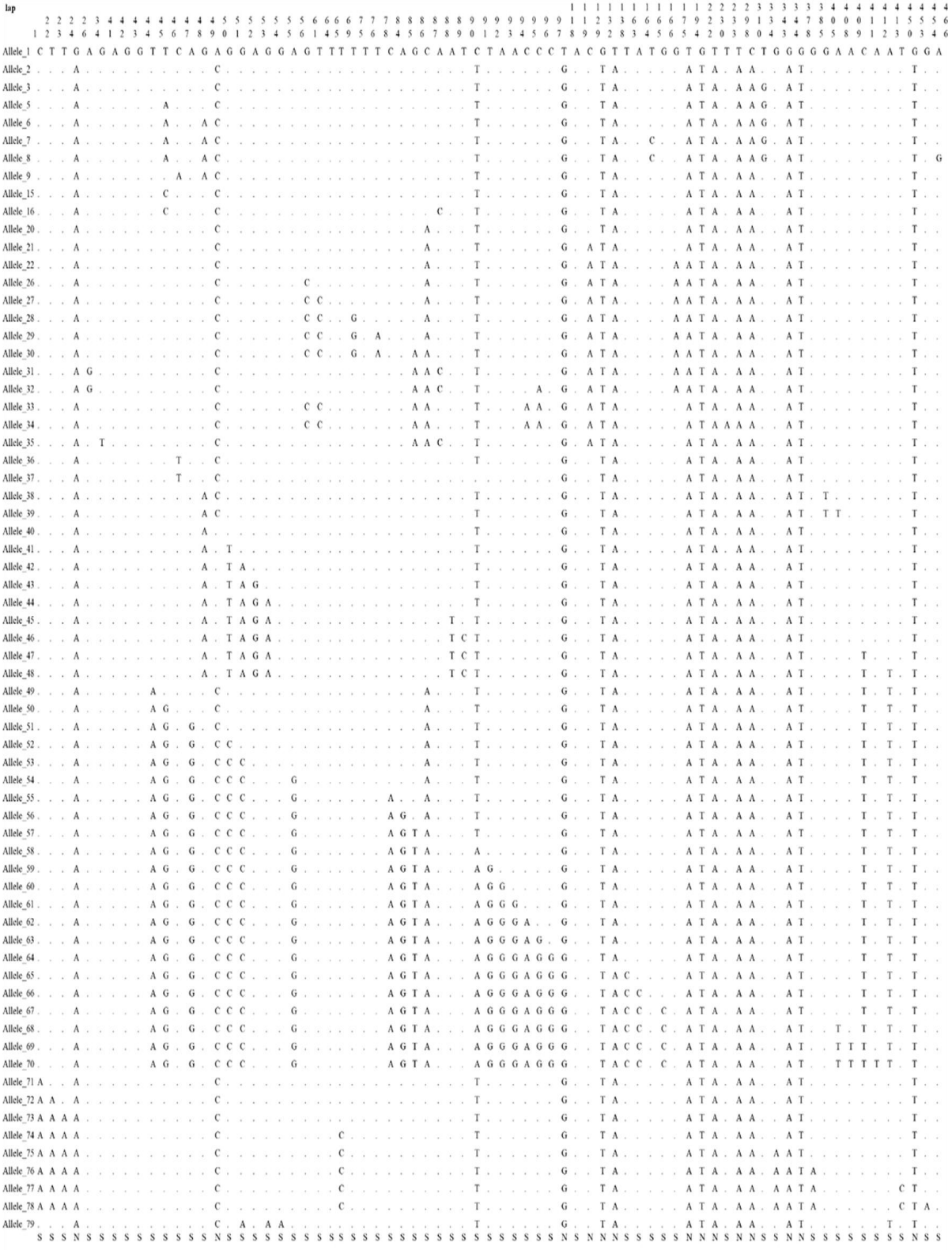


Fig. S1d. Polymorphic sites of *pgm* loci among *V. cholerae* isolates. The nucleotide designation was shown for allele one and for rest of alleles sites only polymorphism is shown along with synonymous and non-synonymous polymorphism.

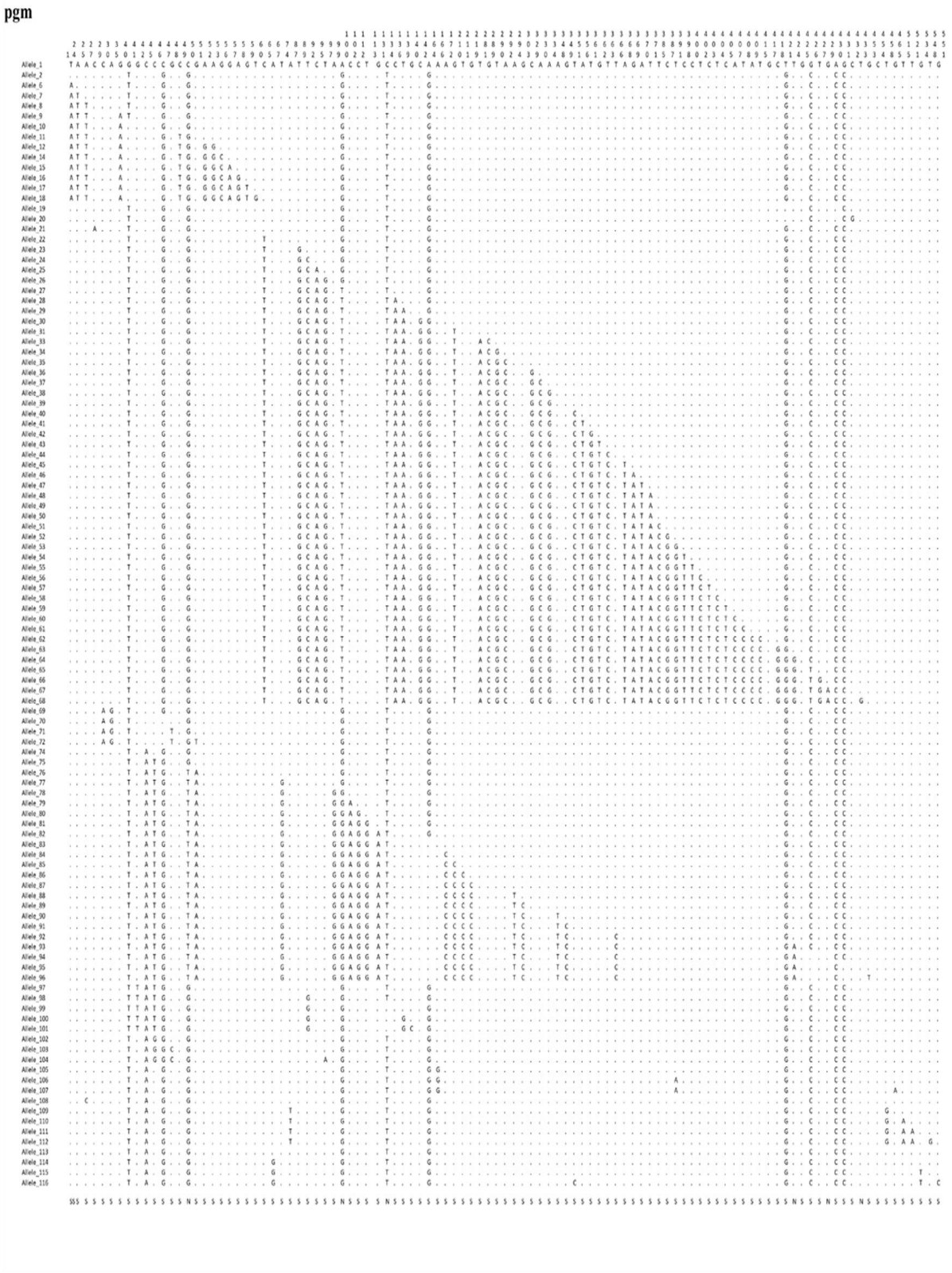
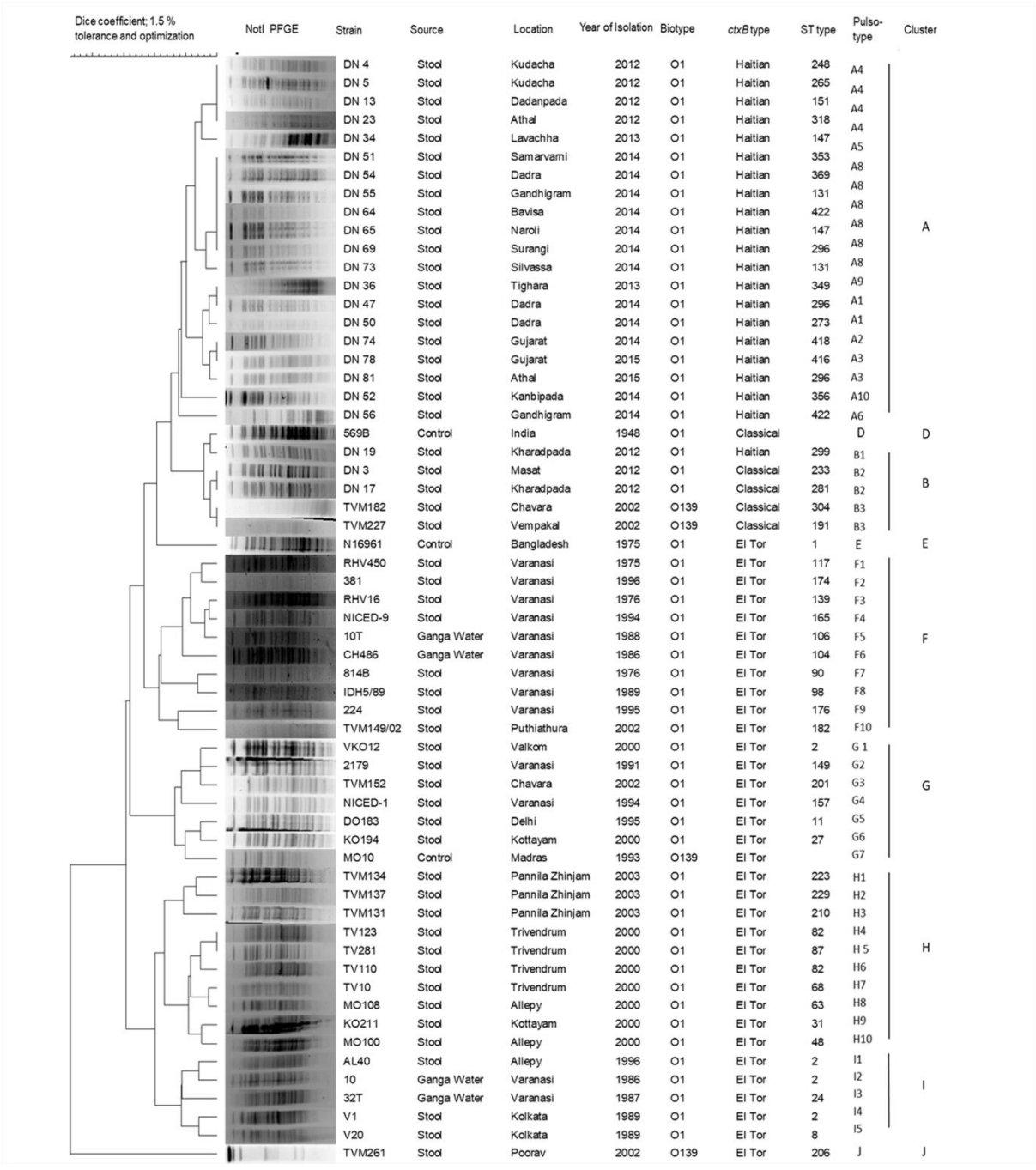


Fig. S1e. Polymorphic sites of *recA* loci among *V. cholerae* isolates. The nucleotide designation was shown for allele one and for rest of alleles sites only polymorphism is shown along with synonymous and non-synonymous polymorphism.

recA

	1	3	5	6	29	44	54	90	98	102	135	136	156	179	183	198	230	252	255	261	264	265	279	287	306	325	339	363	369	383	453	465	479	554	555	558	597	600	608	615	633	640	648	651	655	659	686
Allele_1	T	G	T	G	T	A	A	G	A	A	C	A	G	A	A	T	T	G	C	C	G	A	G	G	T	G	A	A	T	A	T	T	T	G	A	T	G	T	G	T	G	T	C	G	G	A	
Allele_2	G	A	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_4	G	A	A	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_5	G	A	A	A	C	T	G	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_6	G	A	A	A	C	T	G	A	T	G	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_7	G	A	A	A	C	T	G	A	T	G	T	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_8	G	A	A	A	C	T	G	A	T	G	T	G	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_9	G	A	A	A	C	T	G	A	T	G	T	G	A	G	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_10	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_11	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_12	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_13	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_14	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_15	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_16	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_17	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_18	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_19	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_21	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_22	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	.	A	.	.	.	.	T	.	A	.	
Allele_23	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	.	.	.	.	T	.	A	.	
Allele_24	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	C	.	.	.	T	.	A	.	
Allele_25	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	C	C	.	.	T	.	A	.	
Allele_26	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	C	C	.	C	T	.	A	.	
Allele_27	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	C	C	.	C	T	T	A	.	
Allele_28	G	A	A	A	C	T	G	A	T	G	T	G	A	G	T	A	G	A	G	T	.	C	T	C	A	T	G	T	A	T	C	A	G	T	T	G	A	A	C	C	.	C	T	T	A	G	
Allele_35	G	A	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_36	G	C	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_38	G	A	.	A	G	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_39	G	A	.	A	G	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_41	G	A	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	.	A	.	
Allele_42	G	A	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	A	.	T	.	A	.	
Allele_43	G	A	.	A	C	T	.	A	T	.	.	.	A	.	.	A	.	.	.	.	.	.	T	T	A	.	.	T	A	T	.	A	G	.	T	.	.	A	.	.	.	.	T	A	A	.	
	S	N	N	S	S	S	S	N	S	S	S	S	N	S	S	N	S	S	N	S	S	S	S	S	N	S	S	S	N	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	N	S	

Fig. S2. Dendogram presenting the genomic fingerprint pattern of *V. cholerae* O1 and O139 strains isolated from clinical and environmental sources in India. The cluster analysis was carried out using 1.5 % optimization, 1.5 % tolerance and >95 % similarity matrix of Dice similarity coefficient of pulsed-field gel electrophoresis. The dendogram was generated by pulsed-field gel electrophoresis of total chromosomal DNA digested with *NotI* restriction enzyme and correlation between their pulsotype and ST types with respect to their sources and year of isolation.



Strain	Source	Location	Year of Isolation	Biotype	*ctxB* type	ST type	Pulso-type	Cluster
DN 4	Stool	Kudacha	2012	O1	Haitian	248	A4	
DN 5	Stool	Kudacha	2012	O1	Haitian	265	A4	
DN 13	Stool	Dadanpada	2012	O1	Haitian	151	A4	
DN 23	Stool	Athal	2012	O1	Haitian	318	A4	
DN 34	Stool	Lavachha	2013	O1	Haitian	147	A5	
DN 51	Stool	Samarvarni	2014	O1	Haitian	353	A8	
DN 54	Stool	Dadra	2014	O1	Haitian	369	A8	
DN 55	Stool	Gandhigram	2014	O1	Haitian	131	A8	
DN 64	Stool	Bavisa	2014	O1	Haitian	422	A8	
DN 65	Stool	Naroli	2014	O1	Haitian	147	A8	A
DN 69	Stool	Surangi	2014	O1	Haitian	296	A8	
DN 73	Stool	Silvassa	2014	O1	Haitian	131	A8	
DN 36	Stool	Tighara	2013	O1	Haitian	349	A9	
DN 47	Stool	Dadra	2014	O1	Haitian	296	A1	
DN 50	Stool	Dadra	2014	O1	Haitian	273	A1	
DN 74	Stool	Gujarat	2014	O1	Haitian	418	A2	
DN 78	Stool	Gujarat	2015	O1	Haitian	416	A3	
DN 81	Stool	Athal	2015	O1	Haitian	296	A3	
DN 52	Stool	Kanbipada	2014	O1	Haitian	356	A10	
DN 56	Stool	Gandhigram	2014	O1	Haitian	422	A6	
569B	Control	India	1948	O1	Classical		D	D
DN 19	Stool	Kharadpada	2012	O1	Haitian	299	B1	
DN 3	Stool	Masat	2012	O1	Classical	233	B2	
DN 17	Stool	Kharadpada	2012	O1	Classical	281	B2	B
TVM182	Stool	Chavara	2002	O139	Classical	304	B3	
TVM227	Stool	Vempakal	2002	O139	Classical	191	B3	
N16961	Control	Bangladesh	1975	O1	El Tor	1	E	E
RHV450	Stool	Varanasi	1975	O1	El Tor	117	F1	
381	Stool	Varanasi	1996	O1	El Tor	174	F2	
RHV16	Stool	Varanasi	1976	O1	El Tor	139	F3	
NICED-9	Stool	Varanasi	1994	O1	El Tor	165	F4	
10T	Ganga Water	Varanasi	1988	O1	El Tor	106	F5	F
CH486	Ganga Water	Varanasi	1986	O1	El Tor	104	F6	
814B	Stool	Varanasi	1976	O1	El Tor	90	F7	
IDH5/89	Stool	Varanasi	1989	O1	El Tor	98	F8	
224	Stool	Varanasi	1995	O1	El Tor	176	F9	
TVM149/02	Stool	Puthiathura	2002	O1	El Tor	182	F10	
VKO12	Stool	Valkom	2000	O1	El Tor	2	G 1	
2179	Stool	Varanasi	1991	O1	El Tor	149	G2	
TVM152	Stool	Chavara	2002	O1	El Tor	201	G3	G
NICED-1	Stool	Varanasi	1994	O1	El Tor	157	G4	
DO183	Stool	Delhi	1995	O1	El Tor	11	G5	
KO194	Stool	Kottayam	2000	O1	El Tor	27	G6	
MO10	Control	Madras	1993	O139	El Tor		G7	
TVM134	Stool	Pannila Zhinjam	2003	O1	El Tor	223	H1	
TVM137	Stool	Pannila Zhinjam	2003	O1	El Tor	229	H2	
TVM131	Stool	Pannila Zhinjam	2003	O1	El Tor	210	H3	
TV123	Stool	Trivendrum	2000	O1	El Tor	82	H4	
TV281	Stool	Trivendrum	2000	O1	El Tor	87	H 5	H
TV110	Stool	Trivendrum	2000	O1	El Tor	82	H6	
TV10	Stool	Trivendrum	2000	O1	El Tor	68	H7	
MO108	Stool	Allepy	2000	O1	El Tor	63	H8	
KO211	Stool	Kottayam	2000	O1	El Tor	31	H9	
MO100	Stool	Allepy	2000	O1	El Tor	48	H10	
AL40	Stool	Allepy	1996	O1	El Tor	2	I1	
10	Ganga Water	Varanasi	1986	O1	El Tor	2	I2	
32T	Ganga Water	Varanasi	1987	O1	El Tor	24	I3	I
V1	Stool	Kolkata	1989	O1	El Tor	2	I4	
V20	Stool	Kolkata	1989	O1	El Tor	8	I5	
TVM261	Stool	Poorav	2002	O139	El Tor	206	J	J