Supplementary Information for

Genomic attributes of *Vibrio cholerae* O1 responsible for 2022 massive cholera outbreak in Bangladesh

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Supplementary methods

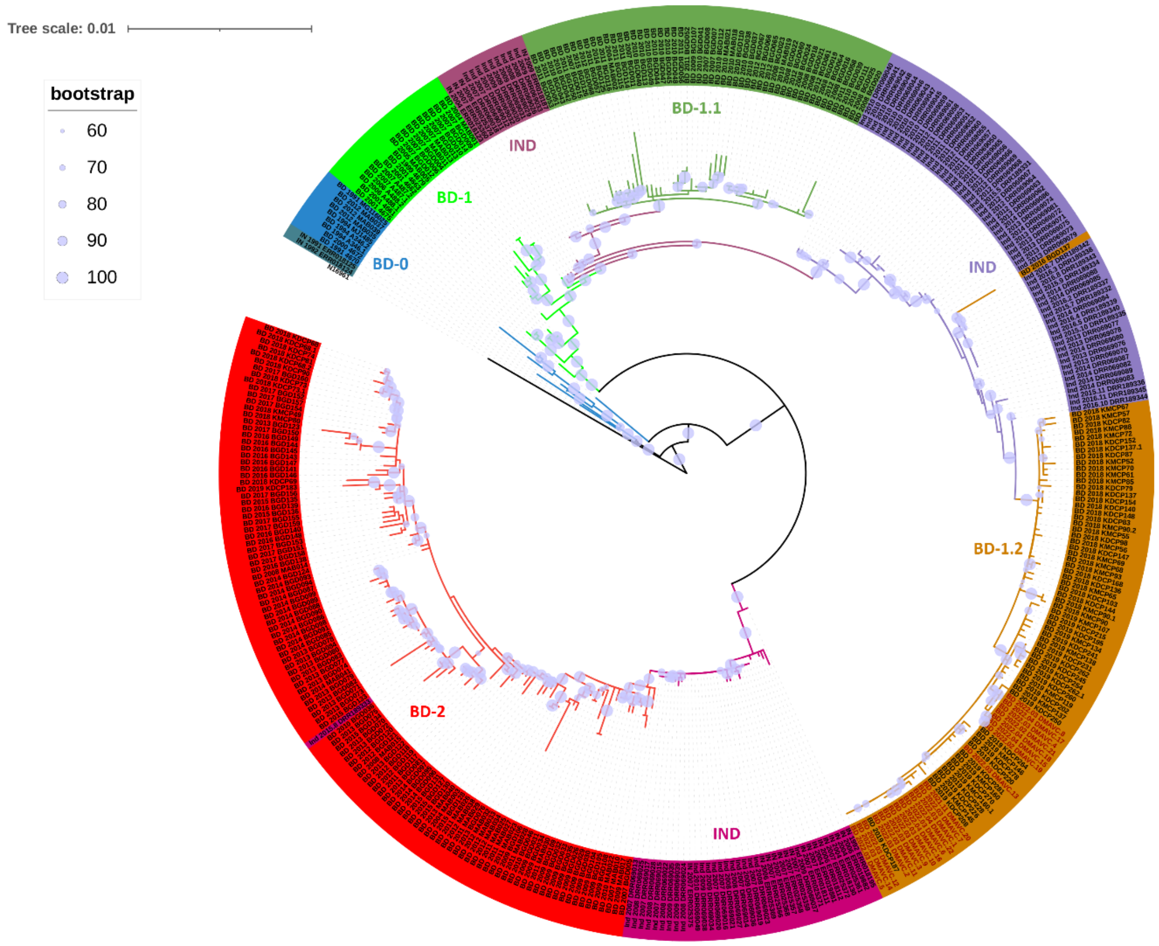
SNP calling

Burrows-Wheeler Alignment tool (BWA)[1] was used to align high-quality reads with reference genome sequence of *V. cholerae* N16961 El Tor (NCBI Accession ID: NC_002505.1 and NC_002506.1) for variant calling. Tool for sequence alignment/map (Samtools v1.9)[2] and Bcftools v1.9[3] were used to call genome variants.

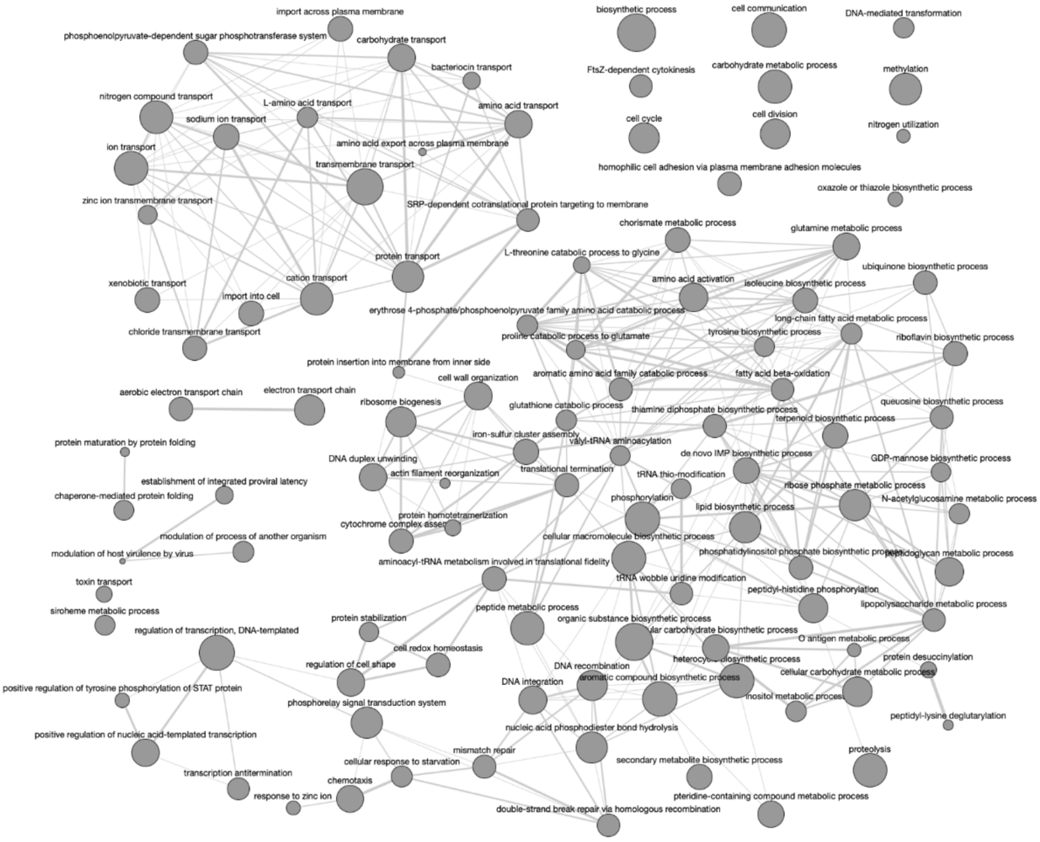
Typing integrating conjugative element

Nucleotide blast command line applications (blastn v2.13.0)[4] was used to match contigs of the strains with seven publicly available sequences of the Integrative and conjugative elements (ICEs)- ICEVchban5 (GQ463140.1), ICEVchind4 (GQ463141.1), ICEVchind5 (GQ463142.1), ICEVchmex1 (GQ463143.1), ICEVflInd1 (GQ463144.1), ICE^{TET} (MK165649.1), and ICE^{GEN} (MK165650.1). Then in-house R-script was used for summarizing the blast results and tabulating best matched ICEs for the strains.

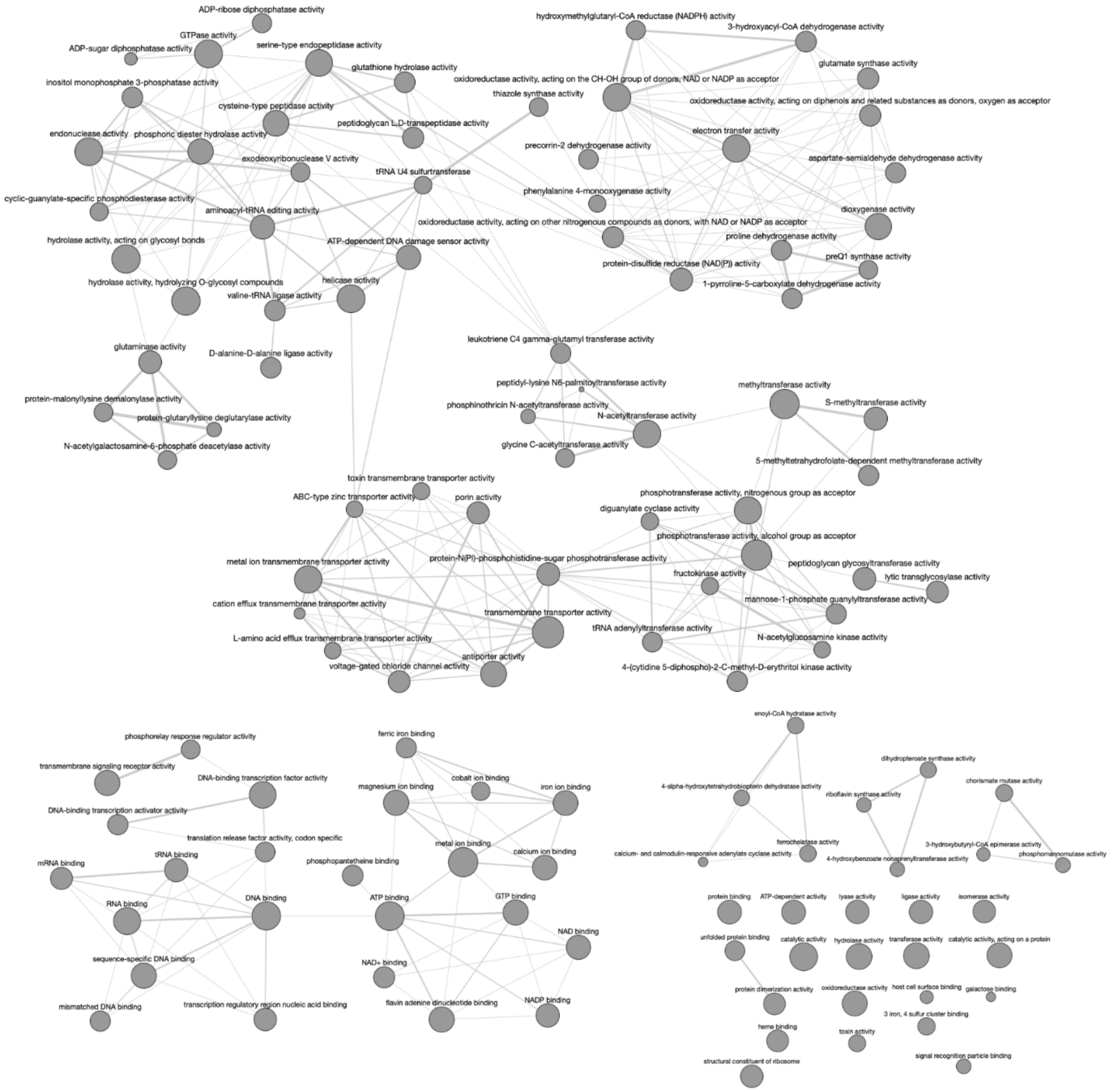
Supplementary figures



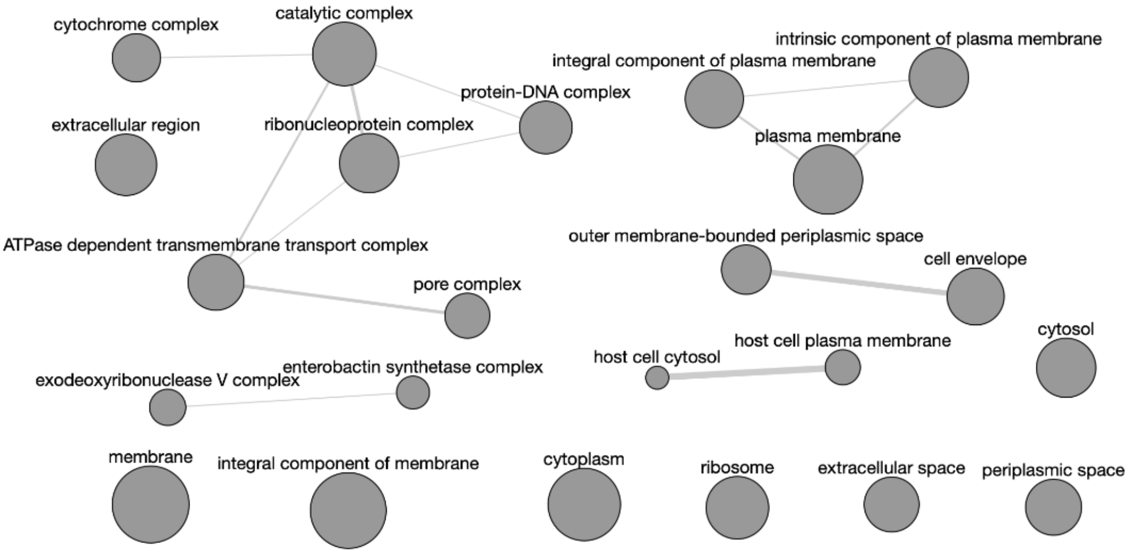
Supplementary Figure 1: Phylogenetic clusters of the *V. cholerae* strains isolated from Bangladesh and India. The greater than 60% bootstrap support value is shown by using a filled circle on the tree. Recently emerged BD-1.2 strains are suggested to have as most recent common ancestor with the strains isolated from India, i.e., predominant in India when BD-2 strains were predominant in Bangladesh. The 22 outbreak strains sequenced in the present study were colored in red, and all belonged to BD-1.2 subclade. Genetic variant data used to construct phylogenetic tree are provided in Source Data 1 and related metadata in Supplementary Data 1.



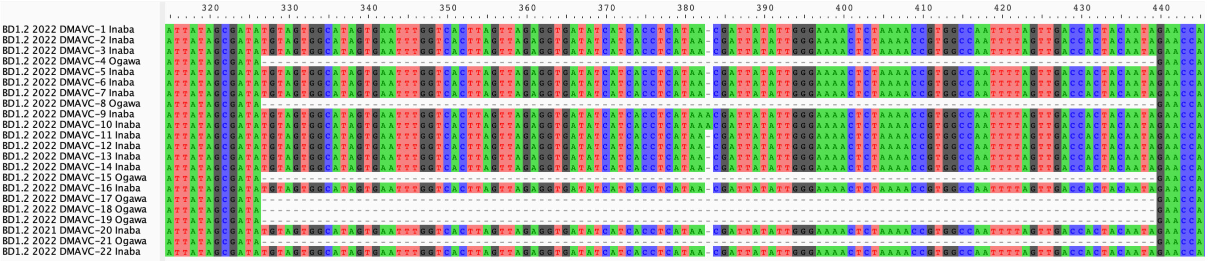
Supplementary Figure 2: Biological process network of lineage associated mutant genes. Complex network of the biological process was found using REVIGO. Ontology of genes were obtained using PANNZER. The GO terms frequencies in the underlying gene ontology annotation are indicated by the bubble size. Highly similar GO terms are connected by edges and the line width indicates the degree of similarity. Gene ontology terms and related descriptions are provided as a Source Data file 4.



Supplementary Figure 3: Molecular functions (MF) network of lineage associated mutant genes. Complex network of important MF was obtained after summarizing the GO terms obtained from PANNZER, that suggest evolution of the bacterium though mutation in the key genes may be fundamental for their fitness and adaptation. Gene ontology terms and related descriptions are provided as a Source Data file 4.



Supplementary Figure 4: Cellular locations of lineage associated mutant gene products. Products of mutant genes locates in cytoplasm, ribosome, plasma membrane, extracellular region, host cell plasma membrane, host cell cytosol, etc. Gene ontology terms and related descriptions are provided as a Source Data file 4.



Supplementary Figure 5: Partial view of *rfbT* gene alignment for the sequenced strains. Within 21 strains isolated in 2022, 7 were Ogawa and 14 were Inaba. All of the Inaba strains had this insertion (2 strains had 113bp insertion and remains 12 had 112bp insertion). In addition, an Inaba strain isolated in 2021 also had 112bp insertion in *rfbT* gene.

Supplementary Tables

Supplementary Table 1. Allelic missense SNP differences between BD-1.1 and BD-1.2 strains

SNP	REF	ALT	Freq BD1.1	Freq BD1.2	Locus tag	Gene	AA change	Product
S1_64791	C	T	0	84	VC_0065	thiG	Ala191Val	Thiazole synthase
S1_93723	G	A	0	84	VC_0094	ubiA	Thr199Ile	4-dydroxybenzoate octaprenyltransferase
S1_148860	C	T	0	84	VC_0157		Cys45Tyr	S8 family peptidase
S1_674880	C	T	0	84	VC_0633	ompU	Gly325Asp	Porin OmpU
S1_1068347	G	A	0	84	VC_0998		Ala1103Thr	Hypothetical protein
S1_1388812	C	T	0	84	VC_1306		Ala447Val	YcjX family protein
S1_1404113	C	T	0	84	VC_1320	rstA	Asp89Asn	Transcriptional regulatory protein
S1_1551891	C	G	0	54	VC_1451	rtxA	Ala582Gly	MARTX multifunctional-autoprocessing repeats-in-toxin holotoxin RtxA
S1_1882714	G	A	0	84	VC_1748		Ser48Asn	Hypothetical protein
S1_2098531	C	A	0	84	VC_1949		His34Asn	L-tyrosine/L-tryptophan isonitrile synthase
S1_2391706	G	A	0	84	VC_2237	mltD	Gly246Asp	Membrane-bound lytic murein transglycosylase D precursor
S1_2405951	A	C	0	84	VC_2251	Skp	Leu46Trp	Chaperone protein Skp precursor
S1_2466598	T	C	0	84	VC_2319	recD	Tyr680Cys	exodeoxyribonuclease V subunit alpha
S1_2577786	C	T	0	84	VC_2407		Arg491His	penicillin-binding protein 3
S1_2873743	C	T	0	84	VC_2701	dsbD	Thr583Ile	protein-disulfide reductase DsbD
S2_398116	G	T	0	84	VC_A0442	aacA4	Gly29Cys	GNAT family N-acetyltransferase
S2_460594	C	T	0	84	VC_A0526	clcA	Gly104Glu	H(+)/Cl(-) exchange transporter ClcA
S2_577065	C	A	0	84	VC_A0640		Ala121Ser	YeeE/YedE family protein
S2_968585	G	A	0	84	VC_A1020	gltR_3	Ala174Val	HTH-type transcriptional regulator GltR
S1_831779	C	A	53	0	VC_0775	vibH	Ala172Ser	norspermidine-2,3-dihydroxybenzoate synthase VibH
S1_1117838	T	G	53	0	VC_1047	fadJ	Val546Gly	fatty acid oxidation complex subunit alpha FadJ
S1_1354024	G	A	53	0	VC_1279	opuD	Ala136Thr	BCCT family transporter
S1_2085882	C	T	53	0	VC_1934	cph2_6	Ala340Val	Phytochrome-like protein cph2
S1_2191730	A	G	53	0	VC_2036	asd1	Val347Ala	aspartate-semialdehyde dehydrogenase
S1_2240207	C	T	53	0	VC_2080	cdhR	Gly52Arg	AraC family transcriptional regulator
S1_2476906	G	A	53	0	VC_2323	tehA	Ala91Val	Tellurite resistance protein TehA
S1_2485904	G	A	53	0	VC_2335		Ala158Thr	gamma-glutamylcyclotransferase
S2_568477	C	T	53	0	VC_A0631		Arg106His	GNAT family N-acetyltransferase
S2_609892	G	A	53	0	VC_A0675	narQ	Arg532Cys	nitrate/nitrite two-component system sensor histidine kinase NarQ

SNPs were labeled according to chromosome name and base pair position. REF = Reference allele, and ALT = alternative allele of the SNPs. Freq_BD-1.1 refers to the frequency BD-1.1 strains were isolated between 2004-2015, and Freq_BD-1.2 refers to the frequency of BD-1.2 strains isolated between 2018-2019. Locus tag are identifiers systematically applied to gene of reference genome N16961 (NCBI database). AA change refer amino acid change.

Supplementary Table 2. Allelic differences in synonymous and intergenic SNPs between BD-1.1 and BD-1.2 strains

SNP/indel	REF	ALT	Freq BD-1.1	Freq BD-1.2	Type	Genes	Nt changes
S1_139591	T	C	0	84	synonymous	ftsX	c.750T>C
S1_454734	G	A	0	84	upstream	arcA_1	c.-571C>T
S1_683129	G	C	0	84	upstream	rimP	c.-222G>C
S1_953331	T	G	0	84	synonymous	thiI	c.390T>G
S1_967866	A	G	0	84	upstream	queF	c.-4229T>C
S1_1429844	T	C	0	84	synonymous	rppH_2	c.1425T>C
S1_1981925	G	A	0	84	synonymous	cydB_1	c.909C>T
S1_2002816	G	A	0	84	synonymous	argT	c.660C>T
S1_2332052	T	C	0	84	downstream	ispE	c.*4260T>C
S1_2484852	C	T	0	84	synonymous	zntB	c.381C>T
S2_350647	G	A	0	84	synonymous	PROKKA_03031	c.252G>A
S2_820659	C	T	0	84	synonymous	PROKKA_03482	c.157C>T
S1_1812498	C	T	53	0	upstream	czcA	c.-3593G>A
S1_1970973	A	G	53	0	synonymous	alaE	c.183T>C

SNPs were labeled according to chromosomal position. For example, "S1_1905668" is an SNP/indel site, where "S" stands for site and "1905668" stands for location of the site base pair. REF = reference allele; and ALT = alternative allele. Freq_BD1.1 = frequency of the BD-1.1 strains isolated between 2004-2015, and Freq_BD1.2 = frequency of the BD-1.2 strains isolated between 2016-2022. Type = type of variant. AA_change = amino acid change.

Supplementary Table 3. Allelic indel differences between BD-1.1 and BD-1.2 strains

SNP/indel	REF	ALT	Freq BD-1.1	Freq BD-1.2	Type	Genes	Nt changes	AA changes
S1_888713	ATTTTTTTT	ATTTTTTTTT	0	84	upstream	ctpH_1	317-316 ins A	NA
S1_890392	TAAAAAA	TAAAAAAA	0	84	upstream	ctpH_1	1994-1993 ins T	NA
S1_2558670	TCCCCC	TCCCCCC	0	84	upstream	carB	1397-1396 ins G	NA
S2_604530	AGAACGAAGATTGC	AC	0	84	Disruptive in-frame deletion	sglT	572-583 del CAATCTTCGTTC	Ala191_Leu195delinsVal
S1_1154205	GAACTATCTCCATAACAAACTATCTCCATAA	GAACTATCTCCATAA	53	0	frameshift	cph2_3	1547-1562 del CAAACTATCTCCATAA	Leu516fs
S2_990917	GCCCC	GCCC	53	0	frameshift	glnP	650 del C	Pro217fs
S1_2961141	CGATCAAGG	CGATCAAGGAGGGTCATTAAATATATAAAGATCAAGG, CGATCAAGGAGGGTCATTAAATATATAAAGATCTATATAGAGATCAAGG	51	0	Feature elongation	NA	NA	NA
S1_2961143	ATCAAGG	ATCAAGGAGGGTCATTAAATATATAAAGATCTATATAGAGATCTTTTCAAGG, ATCAAGG	14	0	Feature elongation	NA	NA	NA

		AGGGTCAAGG						

Insertion-deletions (indels) were named according to chromosomal positions. For example, "S1_1905668" is an SNP/indel site, where "S" stands for site and "1905668" stands for location of the site base pair. REF = reference allele; and ALT = alternative allele. Freq_BD1.1 = frequency of BD-1.1 strains isolated between 2004-2015, and Freq_BD1.2 = frequency of BD-1.2 strains isolated between 2016-2022. Type = type of variant. Nt changes = Nucleotide changes in the protein coding region of a gene, and AA change = amino acid changes.

Supplementary Table 4. Antibiotic resistance gene frequency for global and Asian clade lineages

AMR gene	BD-1 n=20	AS1 n=18	T11 n=5	IND-1 n=15	BD-1.1 n=51	IND-1.1 n=11	T12 n=7	LAT-3 n=7	IND-1.2 n=44	T13 n=24	IND-1.3 n=8	BD-1.2 n=79	AS-2 n=59	IND-2 n=9	BD-2 n=122
varG	20	17	5	15	51	11	7	7	44	24	8	79	59	9	122
catB9	20	18	5	15	51	11	7	7	44	24	8	79	59	9	118
dfrA1	20	18	5	15	51	11	7	7	44	24	8	79	56	7	112
aph(6)-Id	18	18	5	15	51	11	7	7	31	0	8	79	54	7	108
aph(3")-Ib	18	16	5	15	51	11	7	7	31	0	8	79	53	7	108
sul2	17	15	5	15	51	11	7	7	31	0	8	79	54	7	108
floR	18	14	5	15	51	11	7	7	31	0	8	79	12	0	0
tetA(D)	0	2	0	0	0	0	0	0	0	0	0	0	42	7	108
dfrA31	0	0	0	0	0	0	0	0	0	0	0	0	27	4	21
qnrVC1	0	0	0	0	0	0	0	0	0	0	0	0	27	4	21

Here, AMR gene = antimicrobial resistance gene; n = number of strains of the lineages. BD-1, BD-1.1, BD-1.2, and BD-2 are the lineages/groups of strains isolated from Bangladesh; IND-1, IND-1.2, IND-1.3, and IND-2 are the groups of strains isolated from India; AS-1 and AS-2 are groups of the strains isolated from multiple Asian countries; T11, T12, and T13 are African transmission lineages; and LAT-3 is the Latin American transmission lineage.

Supplementary Table 5. Serotypes of Bangladesh strains isolated between April 2017 and March 2022

Year	Month	Total tested strains	Inaba	Ogawa
2017	April	3	3	
	May	19	19	
	June	2	2	
	August	1	1	
	September	3	3	
	October	2	2	
	November	1	1	
	December	1	1	
2018	February	3	3	
	April	4	1	3
	May	2	1	1
	June	1		1
	September	5		5
	October	3		3
	November	1		1
2019	January	2	1	1
	February	1		1
	March	3		3
	April	2		2
	May	3		3
	June	3		2
	July	2		2
	August	3		3
	September	4		4
	October	3		3
	November	2		2
	December	2		2
2020	January	3		3
	February	4		4
	March	4		4
	September	5	2	3
	October	38	32	6
	November	53	53	
	December	10	8	2
2021	January	9	9	
	February	11	11	
	March	21	20	1
	May	3	3	

	June	11	11	
	July	5	5	
	August	6	5	1
	September	12	12	
	October	18	18	
	November	16	16	
	December	9	9	
2022	January	5	3	2
	February	2	1	1
	March	28	20	8

Note: serotypes of 354 strains isolated between April 2017 and March 2022 from Bangladesh that were tested. All tested strains isolated in 2017 were Inaba, as BD-2 strains. From April 2018, Ogawa serotype strains were identified as BD-1.2 strains. From September 2020, Inaba serotype strains of BD-1.2 were detected.

Supplementary Table 6. Serotype and ctxB genotype profiles of randomly chosen strains isolated from clinical samples in Dhaka, Bangladesh, between March and September 2022

SL.	Strain ID	Date	Serotype	*ctx*B-type	Antibiotic Resistance Profile
1	DVC - 638	1/3/22	Ogawa	B7	IPM, SXT, AMP
2	DVC - 640	1/4/22	Inaba	B7	IPM, SXT, AMP
3	DVC - 642	1/5/22	Inaba	B7	SXT, AMP
4	DVC - 644	1/9/22	Inaba	B7	IPM, SXT, AMP
5	DVC - 646	1/10/22	Ogawa	B7	IPM, SXT, AMP
6	DVC - 650	2/6/22	Inaba	B7	SXT
7	DVC - 652	2/6/22	Ogawa	B7	SXT, AMP
8	DVC - 661	3/7/22	Inaba	B7	IPM, SXT, AMP
9	DVC - 663	3/7/22	Ogawa	B7	IPM, SXT, AMP
10	DVC - 664	13/3/2022	Ogawa	B7	IPM, SXT
11	DVC - 667	14/3/2022	Inaba	B7	AMP
12	DVC - 670	27/3/2022	Inaba	B7	IPM, SXT, AMP
13	DVC - 681	4/5/22	Ogawa	B7	IPM, SXT, AMP, AMC
14	DVC - 685	19/4/2022	Inaba	B7	IPM, SXT, AMP
15	DVC - 687	24/4/2022	Inaba	B7	IPM, SXT, AMP
16	DVC - 689	5/10/22	Inaba	B7	SXT, AMP, AMC
17	DVC - 695	16/5/2022	Inaba	B7	IPM, SXT, AMP
18	DVC - 696	17/5/2022	Ogawa	B7	IPM, SXT, AMP
19	DVC - 697	17/5/2022	Inaba	B7	SXT, AMP, AMC
20	DVC - 698	22/5/2022	Ogawa	B7	IPM, SXT, AMP
21	DVC - 700	29/5/2022	Inaba	B7	IPM, SXT, AMP
22	DVC - 702	6/8/22	Ogawa	B7	IPM, SXT, AMP
23	DVC - 705	20/6/2022	Inaba	B7	IPM, SXT, AMP, AMC
24	DVC - 706	20/6/2022	Ogawa	B7	IPM, SXT, AMP
25	DVC - 707	28/7/2022	Ogawa	B7	IPM, SXT, AMP
26	DVC - 709	8/8/22	Ogawa	B7	IPM, SXT, AMP, AMC
27	DVC - 712	21/8/2022	Ogawa	B7	IPM, SXT, AMP, AMC
28	DVC - 715	9/6/22	Ogawa	B7	IPM, SXT, AMP
29	DVC - 719	19/9/2022	Ogawa	B7	IPM, SXT, AMP
30	DVC - 723	27/9/2022	Ogawa	B7	IPM, SXT, AMP

These 30 randomly chosen strains were isolated during and after 2022 massive cholera outbreak in Bangladesh, but they were left out of the sequencing and phylogenetic analysis. According to the ctxB genotype, the randomly chosen strains are likely to belong to the lineage of Asian clade, namely to BD-1.2. Drug Tested - ampicillin (AMP, 10 µg), cefuroxime (CXM, 30 µg), ciprofloxacin (CIP, 5 µg), mecillinam (MEL, 25µg), erythromycin (E, 15 µg), imipenem (IMP, 10 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), tetracycline (TE, 30 µg), aztreonam (ATM, 30 µg), azithromycin (AZM, 15 µg), chloramphenicol (C, 30 µg), and gentamicin (CN, 10 µg).

Supplementary References

1. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
2. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
3. Li, H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27**, 2987 (2011).
4. Quick start - BLAST® Command Line Applications User Manual - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK569856/.