

Supplementary information

***Vibrio* are a potential source of novel colistin-resistance genes in European coastal environments**

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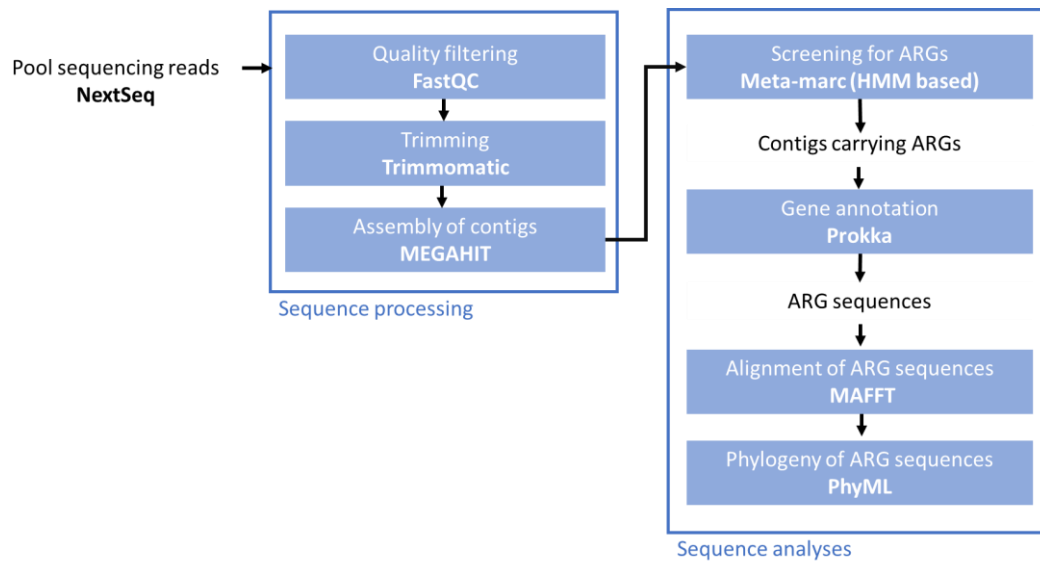


Fig. S1. Bioinformatics analyses for identifying *eptA* and *mcr* gene sequences in Poolseq data.

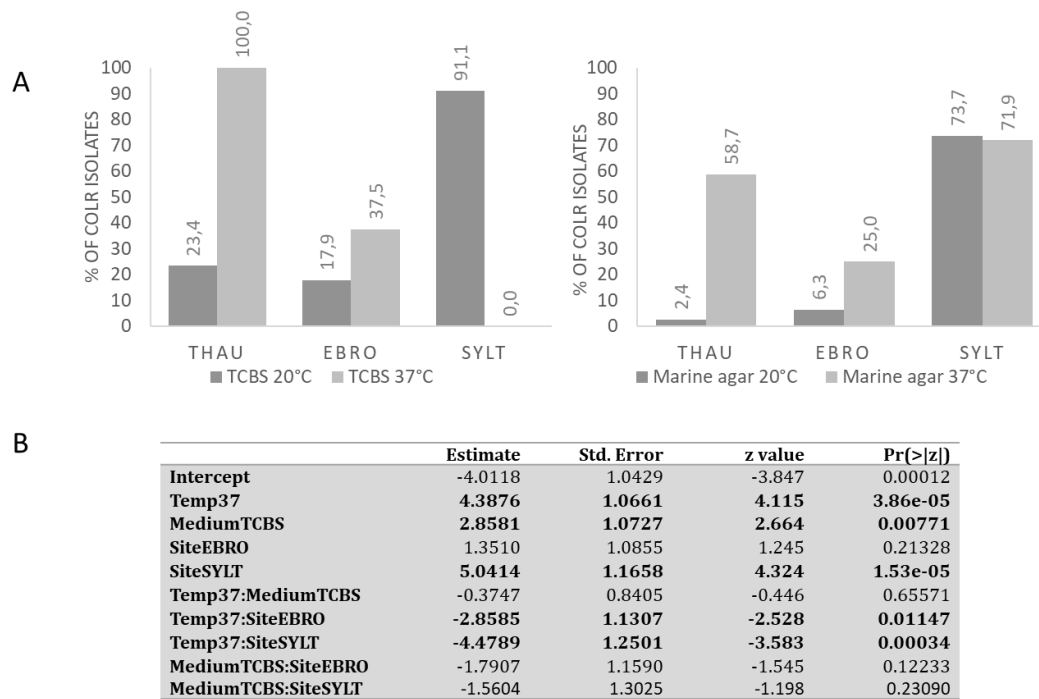


Fig. S2 The percentage of Col-R bacterial isolates across European oyster farms.

(A) % Col-R isolates on TCBS (left panel) or marine agar (right panel). (B) Results of a binomial general linear model showing the effect of environmental origin (Site), temperature (temp) and culture medium (Medium) plus their two-way interactions on the proportion of Col-R bacteria.

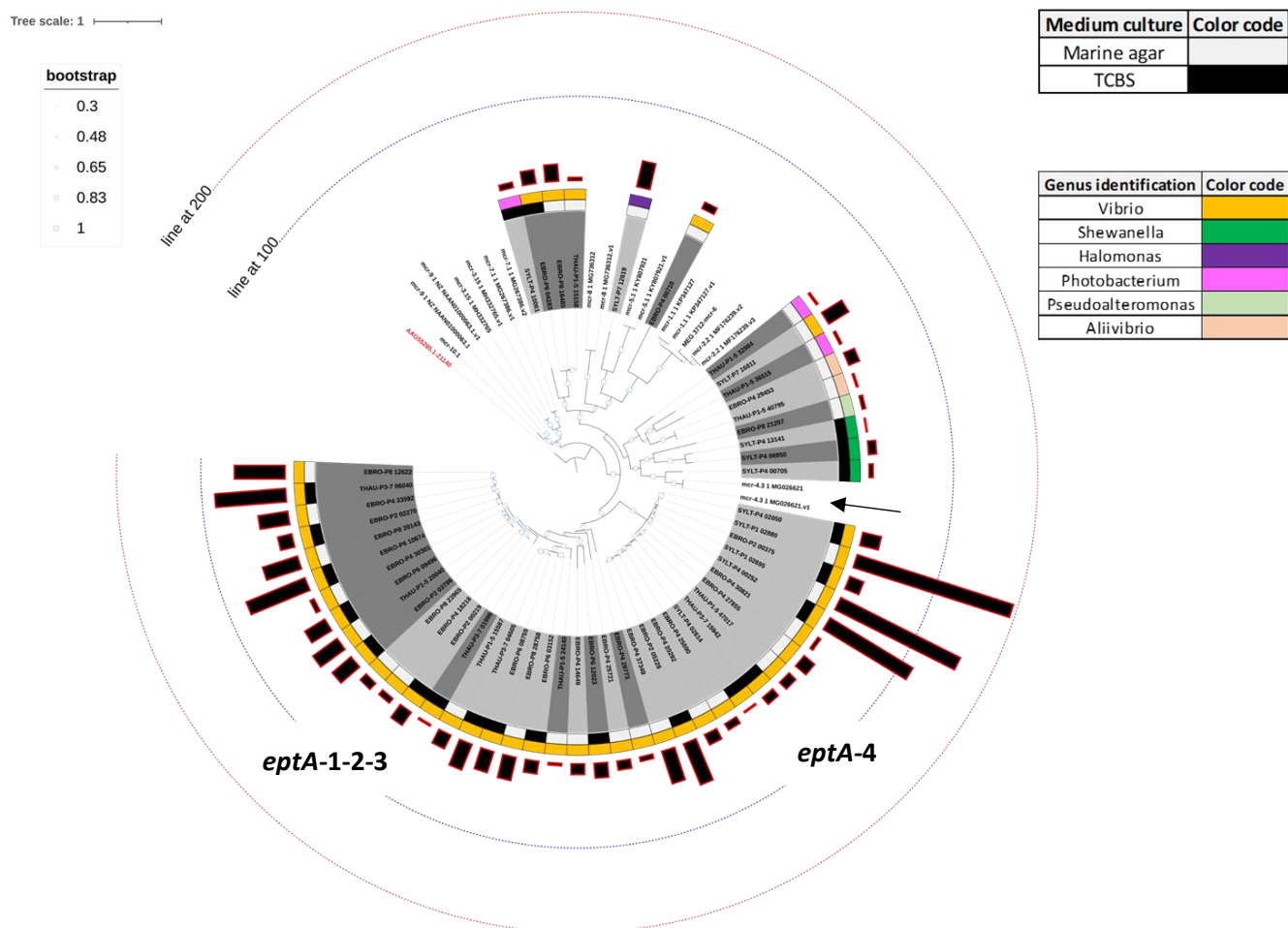


Fig. S3. Phylogeny of EptA/MCR sequences based on medium culture and genus identification.

This figure represents conditions of isolation (back and grey boxes), genus identification (color boxes) and read counts (black bars with a red outline). The phylogenetic tree was generated through maximum likelihood analysis of deduced EptA/MCR amino acid sequences using PhyML V-3.0 with the WAG model and 100 bootstrap runs. Amino acid sequences were extracted from the annotated file generated from pooled sequencing of bacteria isolated from both Marine agar and TCBS medium across three European regions. This figure represents conditions of isolation, genus identification and read counts. Tree was visualized using the iTOL online website. Blue line at 100 and red line at 200 represent the numerical scale of normalization. MCR-4 sequences are indicated by an arrow.

EptA-1	-----MKTIELPTKGLSYVTFLLALYFALVVNIPIYKELVHILTNLDQVKIGFIIT	53
EptA-2	-----MKNITFPYKGISYVTFLLALYFALVVNIPIYKELSHILSNLDEVKIGFIIT	53
EptA-3	-----MKTHNLQKKGISYVTFLLALYFALVVNIPIYKELNGIFSSLDNVKIGFIIT	53
EptA-4	-----MNLPSLNMSINKLPFVLAVYLLVINIPLSLELFSIVQASKSESVAFLIS	50
<i>A. fischeri</i>	MLVLSTLISKIKNNHISMNMVSLITIVSLYFAVAFNYPINEKIYQLSQ---GQEIFLFL	56
<i>V. cholerae</i> El Tor	-----MVIMPTFNKIPFSYLQVLFLAAYFALPLNLPVYAQLAHFNQSTSLDWGFALS	54
<i>V. vulnificus</i>	-----MNRKTLFNRRECSYTFLLVLAIFYFATIVNLPIYKELFSIISHLDSVKVGFVIS	54
<i>V. parahaemolyticus</i>	-----MKTVDSPKKGIPYVAFTVLLALYFALVVNIPIYKELIGIFSCLDEVKIGFIIT	53
	: .:: *: .* *: :: : . * :	
EptA-1	IPIFFFAALNFLNLFNSWPWISKPFIFILLITSGIVSYASNYGTFLDFTDMITNIVETDT	113
EptA-2	IPIFFFAALNFLNLFNSWPWLSKPFIVLLVVSSMVSYASNYGTFLDFTDMITNIVETDT	113
EptA-3	IPIFFLAALNFLNLFNSWPWVGKPFIFILLISSMVSYASNYGTFLDFTDMITNIVETDT	113
EptA-4	IPIFFLAALNFIQVFNWPIFSKPFIFILLITSTLVSYSMFNYGIYVDYGMENVFETNN	110
<i>A. fischeri</i>	TPALLTCAFIIIFSFIAPFYVFKGIVVLTITISAMAFYAALQYNTMFDYAMENIFETNV	116
<i>V. cholerae</i> El Tor	IPLFFLFALNFIQIFSWPYLFKPFFAVLLVLSALISFAGFYQYGVIVDQDMLVNVLETDR	114
<i>V. vulnificus</i>	VPIFFLAALNFLNLFNSWPWLSKPFFAVLLLSAMVSYAGYNYGTFLFDYGMIANIMETDS	114
<i>V. parahaemolyticus</i>	IPIFFFAALNFLNLFNSWPWVGKPFIFILLVVSSLSVYAGYNYGTFLDFTDMITNIVETDT	113
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EptA-1	SEASSYFSAYSLIWTVLMGVLPALLVFKVKFQSLKGNWLRFTLTAKIVSMLASLVIIVAVIA	173
EptA-2	SEASSYLSAYSVIWTLTLLMGMIIPALVFKVKLQPLNGQWLRVLTAKIVSMLASLVIIVAVIA	173
EptA-3	SEASSYLSAYSVIWTLTLLMGVPMIVYKVKLQSQQRHQLLYFSLTKLVSMMLSLAVIAVIA	173
EptA-4	GEAASYVSTHSLLLWLLVMGIPSLILLITKLK--RESWKDFVWKSIGLLSSLIVIAVIA	168
<i>A. fischeri</i>	DEASSYLSASIGYLVFGLIPSLILLKVNIVR-HASWLKELFHRAILMSVAIVGLLLIS	175
<i>V. cholerae</i> El Tor	GEAGSYLTIYSVLWLLGFLVPALALLFTPIRPE-KSALRFLMKKGLSMLSVVVIGVIA	173
<i>V. vulnificus</i>	SEAGSYLSAYSVIWTLTLLMGVPMIVYKVKLQSQQRHQLLYFSLTKLVSMMLSLAVIAVIA	173
<i>V. parahaemolyticus</i>	SEASSYLSAYSVIWTLTLLMGVPMIVYKVKLQSQQRHQLLYFSLTKLVSMMLSLAVIAVIA	173
	.*. *. *: *: : * : * : : : . : : : : : : : *	
EptA-1	GLYYQDYASVGRNNSYLKKIIPQYVYAISYVKEITYLTTPQPYREIGTDAKQSQLALE	233
EptA-2	GLYYQDYASVGRNNSYLKKIIPQYVYSSISYVKAKEYLTTPQPYREIGTDAKQSSALQ	233
EptA-3	SVYYQDYASVGRNNSYLKKIIPQYVYSATGYVKEKYFTTPEPYREIGTDAKQSDSAVK	233
EptA-4	GLFYKDYVSIGRNNSHIKKMIIPTEYVASTVKYINNTRYVKEPIPYQELGLDAQLKPEAK-	227
<i>A. fischeri</i>	VFYFKDYASIGRNNNSYLKMINPAD-AFNTVKYIKNEYLTAPLEYITIGEDAVVTPA---	231
<i>V. cholerae</i> El Tor	GLYYQNYSSVGRNNSYLKKMIIPTEFLYSSFGLVKQRYFTEPMVYQEIIGQDAQKPSAVR	233
<i>V. vulnificus</i>	GLYYQDYASIGRNNNSYLKKMIIPQYVYSATKYVRDITYFSTPQPYRQLGLDAKQSELALQ	233
<i>V. parahaemolyticus</i>	GLYYQDYASVGRNNSYLKKMIIPQYVYSATSYVKENYLTTPQPYREIGTDAKQSSALQ	233
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EptA-1	QAKEKPTLVFVVLGETARTQNYQINGYDRETNPYTSKLDVISFQDVSSCGTA T AVSVPCM	293
EptA-2	QANGKPTLLVFVVLGETARTQNYQLNGYERETNPYTSKLDVVSFKDVASCCTA T AVSVPCM	293
EptA-3	QAQNKPTLLVFVVLGETARTQNYQLNGYKRETNPYTSKLDVISFQEVASCCTA T AVSVPCM	293
EptA-4	-QAEKPTLLVFVVLGETARVYNYQYGYEKEKTAHKTYPNIFFSQVQSCCTA T AVSVPCM	286
<i>A. fischeri</i>	-KNGKPTLMVILVGETARQNSAYNGYERETNPYTKDLGLITFQNVTSCTA T AHSLPCM	290
<i>V. cholerae</i> El Tor	QATQKPTLVFVVLGETARVYNYQYLYGPRDTNAYTAPFQPIFFKDVASCCTA T AVSVPCM	293
<i>V. vulnificus</i>	QADQKPTLLVFVVLGETARSQNYQANGYARPTNQYTNELGMISFQDVSSCGTA T AVSVPCM	293
<i>V. parahaemolyticus</i>	QAQDKPTLLVFVVLGETARTQNYQLNGYERETNPYTSKLDVISFQDVASCCTA T AVSVPCM	293
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EptA-1	FSQLPRTDFDRSTADNQDNALDIMQRAGIDLMMWKDNDGGDKGVAHKINKMDVDRSQKNEQ	353
EptA-2	FSQLTRSDFRDRSRADNQDNALDIMQRAGVDLLWKENDGGDKGVAHNITKIEVDRSQKNEQ	353
EptA-3	FSQLTRSEFERTKADNQDNALDIMQRAGVDLIWKENDGGDKGVAHKIKKIEVNRKQTSPL	353
EptA-4	FSNMNRSNYDRDKAYNQDNVVDIMNRAGIHSIWREHDGGDKAVAHRIKEMTLVAKDSDDL	346
<i>A. fischeri</i>	FSNMTRTSYNKDRANNQDNALDVLTHAGVKAVWIDNDGGDKAVAKNIEKEMITDHSNSL	350
<i>V. cholerae</i> El Tor	FSNMNRQNFDRSRADNQDNVLDILQRAGISLLWKENDGGDKNVAKNIPLKELARDNREGI	353
<i>V. vulnificus</i>	FSNMTRSNFDRPTADNQDNALDILNRAGVSLWKENDGGDKDVAKNIPLFTVDRSRKDAL	353
<i>V. parahaemolyticus</i>	FSQLTRNQFDRKQADNQDNALDIMQRAGIDLWKENDGGDKGVAHKIKKIEVDRKQONAL	353
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EptA-1	CNGSTCYDIALNNLDDQIASMGKNRLVALHLIGSHGPTYFYQRYPKDKAFFQPCPRADI	413
EptA-2	CNGSTCYDMALLNNLDTQISSMGKNRVIAMHLIGSHGPTYFYQRYPKDKAFFQPCPRADI	413
EptA-3	CNGQTCYDMALLDHFQDEADMHGKNRVVAMHLIGSHGPTYFYQRYPRDKAFFQPCPRADI	413
EptA-4	CNNNVCYDTAMLENFEQDTQDLKQDSIIIFYHISGSHGPTYFYERYPDHKKFTPDARADI	406
<i>A. fischeri</i>	CNGSSCYDEILLKGLDKRIAEQGNQVYALHMGSHGPTYFYKRYPKEMAVFGPACNRSDI	410
<i>V. cholerae</i> El Tor	CDGDTCYDIAMLENLDQEIATQQGNRMIFMHFIGSHGPTYFYKRYPKEMAVYQPCPRADI	413
<i>V. vulnificus</i>	CNGSTCYDMALLNFQDVENLKGNRVLAHLIGSHGPTYFYQRYPKDKAAFFMPDCPRADI	413
<i>V. parahaemolyticus</i>	CNGQTCYDMALLSDFQEVSNMGNRVVAMHLIGSHGPTYFYQRYPKDKAFFQPCPRADI	413
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EptA-1	ENCSVEQIVNTYDNTIRYTDVFLDQTI AKLKALEDKYNTAMIYVSDHGESLGENGLFLHG	473
EptA-2	ENCSVEQIVNTYDNTIRYTDVFLDQTI AKLKLTLEDKYNTAMIYVSDHGESLGENGLFLHG	473
EptA-3	ENCSVEQIVNTYDNTIRYTDVFLAETINKLKALESQYNTALIYVSDHGESLGENGLFLHG	473
EptA-4	ENCTKEEVNTYDNTILYTDFFLSQAIQKLEKLTDKYNVALMYISDHGESLGENGVYLHG	466
<i>A. fischeri</i>	ENCSDAEITNVYDNTLVYTDYVIAQTVKKLQSYSDKYNVVMYIISDHGESLGENGLYLHG	470
<i>V. cholerae</i> El Tor	ENCSVEQIVNTYDNTIRYSDYVMSQLLAKLDSLQDRYNTALIYISDHGESLGENGLFLHG	473
<i>V. vulnificus</i>	ENCSVEQIVNTYDNTILYTDYVLSQTINKLKALEERYNTALIYVSDHGESLGENGLFLHG	473
<i>V. parahaemolyticus</i>	ENCSVEEIVNTYDNTIRYTDVLEQTINKLKLTLEDKYNTALIYVSDHGESLGESEGMFLHG	473
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EptA-1	MPYGLAPDFQKRVPMILWMSPGFKQAKHINTDCLEQEAQQTATHSHDNVFSLLGIMDVE	533
EptA-2	MPYGLAPDFQKRVPMMLWMSPGFKQAKHINSCLQQAQQTATHSHDNVFSLLGIMDIE	533
EptA-3	MPYGLAPDFQKRVPLLMWLSSGFKQVKQINTDCLNEAQKAGQYSHDNVFSLLGVMVQ	533
EptA-4	MPYSLAPKEQTHVPMILWMSDGFQAAQKRISSETCLRKAG-KEQSFSHDNLFDSSLGLMDVQ	525
<i>A. fischeri</i>	APYMMAPKEQTHVPWYIWMSSDDYANQKIDKAAVIDNS-ATGDYSHDNLFHTILGLYGV	529
<i>V. cholerae</i> El Tor	MPYSLAPEYQTRVPLLIWMSGFSQSKGIDVECLRSNS--ELPYAHQNLFSLLGVMVVS	531
<i>V. vulnificus</i>	MPYSLAPEYQTKVPLMLWMSPGFAQAKQINADCLKQAKQTDYSHDYIFHSSLGVMNVS	533
<i>V. parahaemolyticus</i>	MPYGLAPDFQKRVPLIMWMSPSFKQAKHINTDCLSKAQNAGKYSHDNVFSLLGIMDVK	533
	** :*. *.:** :*: * .: * * . .: . . . :*: :*.:**: .:	
EptA-1	TDAYDGDLDIFKTCRTS--	550
EptA-2	TNAYDGKLDIFKTCRNS--	550
EptA-3	TQAYDGKLDIFKDCRTS-	551
EptA-4	TNEYRASQDIFAPCR----	540
<i>A. fischeri</i>	TKAKDNALDIMAN-----	542
<i>V. cholerae</i> El Tor	TKAYQANLDFAKCRTSQS	550
<i>V. vulnificus</i>	TSEYDATLDFKQCRAQ--	550
<i>V. parahaemolyticus</i>	TQAYDGQLDIFKTCRTVS-	551
	*. *::	

Fig. S4. Multiple sequence alignment of EptA-1 to -4 with EptA orthologs in *Vibrionaceae*.

The alignment was performed using Clustal Omega (1.2.4). The conserved catalytic threonine residue (T) is in shown in red. EptA-1 to -4 sequences were chosen as one representative of each clade (amino acid sequences deduced from poolseq data). The amino acid sequences from previously characterized EptA orthologs were obtained in NCBI database and the accession numbers are: AAW87280.1 (*A. fischeri* ES114), AAF96994.1 (*V. cholerae* O1 biovar El Tor str. N16961), VVM06_RS15980 (*V. vulnificus* MO6-24/O), and BAC62623.1 (*V. parahaemolyticus* RIMD 2210633).

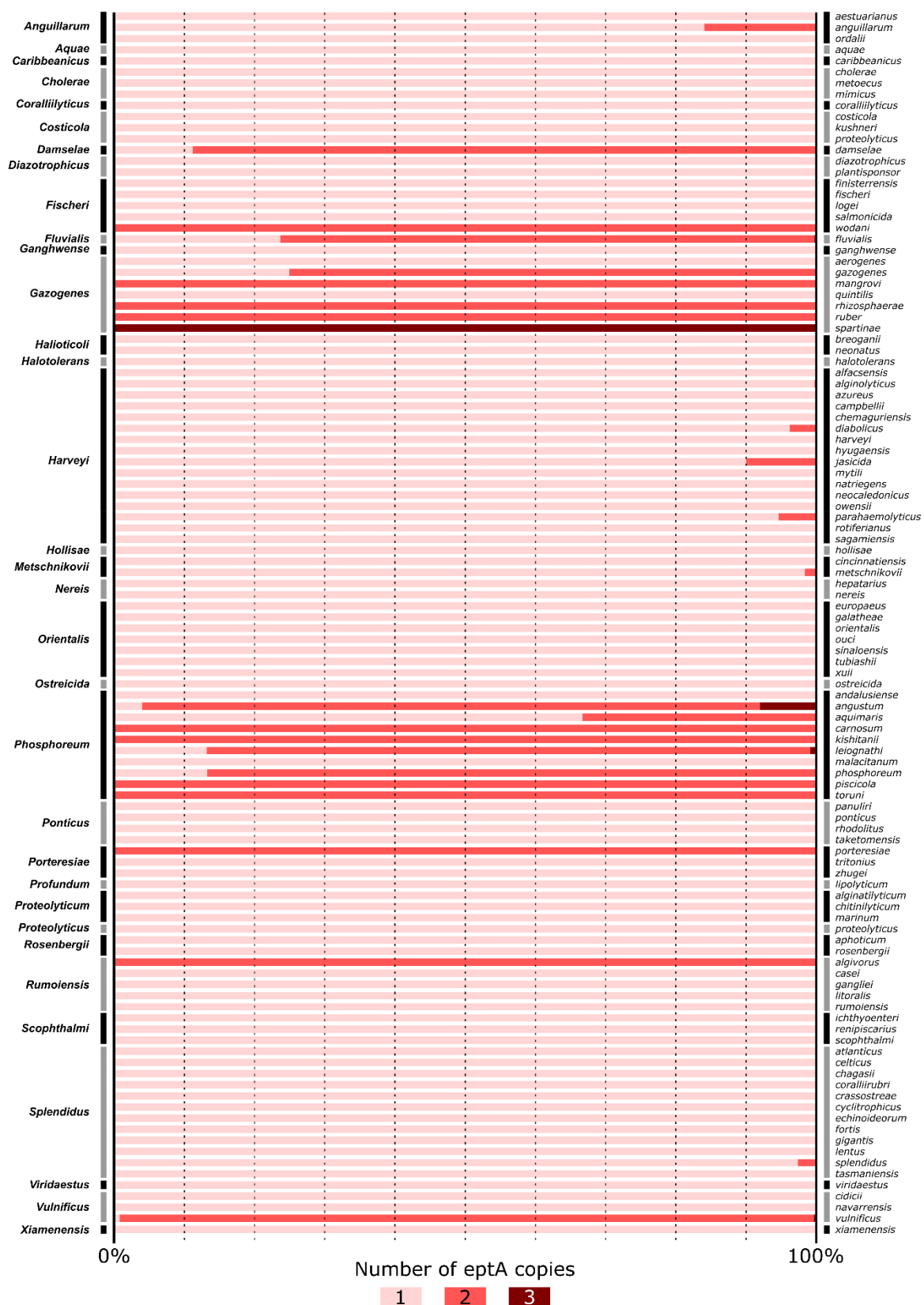


Fig. S5. Percentage of *eptA* paralogues in species of *Vibrionaceae*. Species of *Vibrionaceae* (right) are sorted by clade (left). The color code indicates the number of *eptA* copy per genome: pink (1 gene copy), dark pink (two gene copies), brown (3 gene copies). The scale bar indicates the percentage of genomes carrying 1, 2 or 3 *eptA* copies in each species.

Vibrio sp. TH21_20A_OC-7 – chromosome I – *arn* locus – 7.8 kb



Vibrio sp. TH21_20A_OC-7 – chromosome I – *phoP phoQ* locus – 2.1 kb



Fig. S6. Alternative colistin resistance mechanisms identified in *Vibrio* strains TH21_20A_OC7, TH21_20_OH4, and TH21_20A_OG1.

Each of these isolates contained both *arnBCADTEF* and *phoP/Q* genes within their genomes, although they were situated in distinct genomic regions.

Table S1. Metadata on *mcr/eptA* unique sequences found in Pool sequencing data

Sampling sites	GPS coordinates	Sampling date	Culture medium	Temp	No. CFU	Bacterial Pool ID	Total sequenced reads	Accession number	No. unique <i>mcr/eptA</i> sequences total (<i>mcr</i> ; <i>eptA</i>)
THAU	N: 43°26.058' E: 003°39.878'	18/10/2021	Marine agar	37°C	48	THAU-P1_5	7,53E+07	SAMN37810832	7 (1;6)
				20°C	44				
			TCBS	37°C	6	THAU-P3_7	8,38E+07	SAMN37810833	4 (0;4)
				20°C	48				
EBRO	N: 40°37.106880' E: 0°37.318320'	25/10/2021	Marine agar	37°C	48	EBRO-P8	3,59E+07	SAMN37810834	6 (2;4)
				20°C	47	EBRO-P4	6,88E+07	SAMN37810835	13 (1;12)
			TCBS	37°C	18	EBRO-P6	1,28E+07	SAMN37810836	6 (1;5)
				20°C	36	EBRO-P2	1,44E+07	SAMN37810837	5 (0;5)
SYLT	N: 55° 1' 42.539" E: 8° 26' 1.953"	10/01/2022	Marine Agar	37°C	32	SYLT-P7	3,05E+07	SAMN37810838	2 (0;2)
				20°C	19	SYLT-P1	6,57E+06	SAMN37810839	2 (0;2)
			TCBS	20°C	48	SYLT-P4	7,73E+07	SAMN37810840	6 (3;3)
				37°C	0	na	na	na	0 (0;0)
Total					394				51 (8;43)

Table S2. List of the 51 *mcr/eptA* unique sequences found in pool sequencing data

Gene name	<i>mcr/eptA</i> sequence ID	Genbank ID	From poolseq library	Sampling site	Genetic environment	Genus based on BlastP best hit	Culture medium	Temp	No. of mapped Reads	Normalised read counts * 10 ⁶
<i>eptA-1</i>	EBRO-P8_12622	OR578988	EBRO-P8	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	37°C	2136	60,39
<i>eptA-1</i>	THAU-P3-7_06040	OR578979	THAU-P3-7	THAU	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	20+37°C	7010	84,39
<i>eptA-1</i>	EBRO-P2_02270	OR578984	EBRO-P2	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	20°C	247	17,4
<i>eptA-1</i>	EBRO-P4_33592	OR578987	EBRO-P4	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	20°C	2440	35,81
<i>eptA-1</i>	EBRO-P2_03786	OR578981	EBRO-P2	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	20°C	418	29,44
<i>eptA-1</i>	EBRO-P4_30301	OR578985	EBRO-P4	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	20°C	375	5,5
<i>eptA-1</i>	EBRO-P6_09496	OR578982	EBRO-P6	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	37°C	356	28,11
<i>eptA-1</i>	THAU-P1-5_20040	OR578980	THAU-P1-5	THAU	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	20+37°C	2669	35,89
<i>eptA-1</i>	EBRO-P8_20143	OR578986	EBRO-P8	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	37°C	1546	43,71
<i>eptA-1</i>	EBRO-P6_10674	OR578983	EBRO-P6	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	37°C	958	75,63
<i>eptA-2</i>	EBRO-P2_00219	OR578989	EBRO-P2	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	20°C	274	19,3
<i>eptA-2</i>	EBRO-P4_18218	OR578990	EBRO-P4	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	20°C	492	7,22
<i>eptA-2</i>	EBRO-P8_23965	OR578991	EBRO-P8	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	37°C	359	10,15
<i>eptA-3</i>	EBRO-P8_28758	OR578996	EBRO-P8	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	37°C	1005	28,41
<i>eptA-3</i>	EBRO-P6_08759	OR578995	EBRO-P6	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	37°C	333	26,29
<i>eptA-3</i>	EBRO-P6_03152	OR578994	EBRO-P6	EBRO	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	37°C	195	15,4
<i>eptA-3</i>	THAU-P1-5_15587	OR578993	THAU-P1-5	THAU	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	MA	20+37°C	1356	18,23
<i>eptA-3</i>	THAU-P3-7_04605	OR578992	THAU-P3-7	THAU	<i>rstA-rstB-Zipper-dgkA-eptA</i>	<i>Vibrio</i>	TCBS	20+37°C	2751	33,12
<i>eptA-4</i>	EBRO-P2_00375	OR579005	EBRO-P2	EBRO	<i>eptA</i>	<i>Vibrio</i>	TCBS	20°C	256	18,03
<i>eptA-4</i>	SYLT-P4_02614	OR578999	SYLT-P4	SYLT	<i>eptA</i>	<i>Vibrio</i>	TCBS	20°C	156	2,03
<i>eptA-4</i>	EBRO-P4_30821	OR579010	EBRO-P4	EBRO	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	288	4,23
<i>eptA-4</i>	SYLT-P1_02695	OR579002	SYLT-P1	SYLT	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	1017	159,08
<i>eptA-4</i>	SYLT-P4_00252	OR579000	SYLT-P4	SYLT	<i>eptA</i>	<i>Vibrio</i>	TCBS	20°C	8823	115,02
<i>eptA-4</i>	EBRO-P2_05228	OR579004	EBRO-P2	EBRO	<i>eptA</i>	<i>Vibrio</i>	TCBS	20°C	753	53,04
<i>eptA-4</i>	EBRO-P4_25590	OR579006	EBRO-P4	EBRO	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	612	8,98
<i>eptA-4</i>	EBRO-P4_20292	OR579007	EBRO-P4	EBRO	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	946	13,89

eptA-4	EBRO-P4_37349	OR579008	EBRO-P4	EBRO	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	2770	40,66
eptA-4	SYLT-P4_02050	OR579001	SYLT-P4	SYLT	<i>eptA</i>	<i>Vibrio</i>	TCBS	20°C	1745	22,75
eptA-4	THAU-P3-7_15942	OR578997	THAU-P3-7	THAU	<i>eptA</i>	<i>Vibrio</i>	TCBS	20+37°C	891	10,73
eptA-4	EBRO-P4_27855	OR579009	EBRO-P4	EBRO	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	1117	16,4
eptA-4	SYLT-P1_02880	OR579003	SYLT-P1	SYLT	<i>eptA</i>	<i>Vibrio</i>	MA	20°C	1244	194,59
eptA-4	THAU-P1-5_47017	OR578998	THAU-P1-5	THAU	<i>eptA</i>	<i>Vibrio</i>	MA	20+37°C	857	11,52
other eptA	SYLT-P7_16811	OR579015	SYLT-P7	SYLT	<i>eptA-dgkA</i>	<i>Vibrio</i>	MA	37°C	849	28,11
other eptA	EBRO-P4_25721	OR579018	EBRO-P4	EBRO	<i>dgkA-eptA</i>	<i>Vibrio</i>	MA	20°C	753	11,05
other eptA	THAU-P1-5_24140	OR579014	THAU-P1-5	THAU	<i>dgkA-eptA</i>	<i>Vibrio</i>	MA	20+37°C	226	3,04
other eptA	THAU-P3-7_01996	OR579013	THAU-P3-7	THAU	<i>rstA-rstB-Zipper-eptA</i>	<i>Vibrio</i>	TCBS	20+37°C	192	2,31
other eptA	THAU-P1-5_40795	OR579012	THAU-P1-5	THAU	<i>eptA-arlR</i>	<i>Aliivibrio</i>	MA	20+37°C	177	2,38
other eptA	EBRO-P4_14649	OR579020	EBRO-P4	EBRO	<i>Zipper-eptA</i>	<i>Vibrio</i>	MA	20°C	883	12,96
other eptA	THAU-P1-5_36515	OR579011	THAU-P1-5	THAU	<i>eptA-PGT1-arlR-sasA</i>	<i>Photobacterium</i>	MA	20+37°C	230	3,09
other eptA	EBRO-P4_29453	OR579016	EBRO-P4	EBRO	<i>eptA</i>	<i>Aliivibrio</i>	MA	20°C	953	13,99
other eptA	SYLT-P7_12619	OR579022	SYLT-P7	SYLT	<i>dgkA-hpap2-eptA</i>	<i>Halomonas</i>	MA	37°C	954	31,58
other eptA	EBRO-P6_12023	OR579019	EBRO-P6	EBRO	<i>dgkA-eptA</i>	<i>Vibrio</i>	TCBS	37°C	225	17,76
other eptA	EBRO-P4_29773	OR579017	EBRO-P4	EBRO	<i>dgkA-eptA-PGT1</i>	<i>Vibrio</i>	MA	20°C	373	5,47
other mcr	EBRO-P4_00710	OR579026	EBRO-P4	EBRO	<i>mcr</i>	<i>Vibrio</i>	MA	20°C	580	8,51
other mcr	SYLT-P4_10061	OR579023	SYLT-P4	SYLT	<i>mcr-dgkA</i>	<i>Photobacterium</i>	TCBS	20°C	517	6,74
other mcr	SYLT-P4_06950	OR579024	SYLT-P4	SYLT	<i>mcr</i>	<i>Shewanella</i>	TCBS	20°C	754	9,83
other mcr	EBRO-P6_04283	OR579027	EBRO-P6	EBRO	<i>mcr-dgkA</i>	<i>Vibrio</i>	TCBS	37°C	211	16,66
other mcr	THAU-P1-5_15158	OR579021	THAU-P1-5	THAU	<i>mcr-dgkA</i>	<i>Vibrio</i>	MA	20+37°C	292	3,93
other mcr	EBRO-P8_21207	OR579029	EBRO-P8	EBRO	<i>mcr-dgkA</i>	<i>Pseudoalteromonas</i>	MA	37°C	154	4,35
other mcr	SYLT-P4_00705	OR579025	SYLT-P4	SYLT	<i>mcr</i>	<i>Shewanella</i>	TCBS	20°C	386	5,03
other mcr	EBRO-P8_16485	OR579028	EBRO-P8	EBRO	<i>mcr-dgkA</i>	<i>Vibrio</i>	MA	37°C	721	20,38

Table S3. Amino acid sequence identity between EptA variants

[illegible]

% identity

40	50	60	70	80	90	##
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Table S4. Strains and Plasmids

Bacterial strains	Superclade	Isolation Source	Temp	Description	Reference	Accession Number	Conting number	size (Mb)	CDSs	Coverage
<i>Vibrio harveyi</i> Th15_Z_F11	Harveyi	Water column, Thau lagoon, France	20°C	eptA-1; [Col-R]	(Oyanedel et al., 2023)	ERS9919775	53	5,7	5395	
<i>Vibrio harveyi</i> Th15_F5_F11	Harveyi	Water column, Thau lagoon, France	20°C	eptA-1; [Col-R]	(Oyanedel et al., 2023)	ERS9919777	106	5,9	5524	
<i>Vibrio jasicida</i> Th15_F5_H11	Harveyi	Water column, Thau lagoon, France	20°C	eptA-1; [Col-R]	(Oyanedel et al., 2023)	ERS9919787	52	6,1	5712	
<i>Vibrio owensii</i> Th15_Z_G08	Harveyi	Water column, Thau lagoon, France	20°C	eptA-1; [Col-R]	(Oyanedel et al., 2023)	ERS9919782	98	6,0	5566	
<i>Vibrio rotiferianus</i> Th15_O_H08	Harveyi	Oyster, Thau lagoon, France	20°C	eptA-2; [Col-R]	(Oyanedel et al., 2023)	ERS9919779	81	5,2	4835	
<i>Vibrio rotiferianus</i> Th15_O_G05	Harveyi	Oyster, Thau lagoon, France	20°C	eptA-2; [Col-R]	(Oyanedel et al., 2023)	ERS9919778	85	5,3	4956	
<i>Vibrio splendidus</i> 7T7_2	Harveyi	Oyster, Bay of Brest, France	20°C	eptA-4; [Col-S]	(Bruto et al., 2016)		297	5,9	5594	
<i>Vibrio crassostreae</i> J2-4	Splendidus	Oyster, Bay of Brest, France	20°C	eptA-4; [Col-S]	(Lemire et al., 2015)	PRJEB5888	84	5,7	5285	
<i>Vibrio crassostreae</i> J2-14	Splendidus	Oyster, Bay of Brest, France	20°C	eptA-4; [Col-S]	(Lemire et al., 2015)	PRJEB5892	65	5,7	5244	
<i>Vibrio crassostreae</i> J5-15	Splendidus	Oyster, Bay of Brest, France	20°C	eptA-4; [Col-S]	(Lemire et al., 2015)	PRJEB5879	92	5,7	5345	
<i>Vibrio sp.</i> TH21_20_OG1	unknown	Oyster, Thau lagoon, France	20°C	eptA-, mcr-; [Col-R]	this study	ERR12116512	56	5,4	4945	78X
<i>Vibrio sp.</i> TH21_20_OH4	unknown	Oyster, Thau lagoon, France	20°C	eptA-, mcr-; [Col-R]	this study	ERR12116510	76	5,4	4903	34X
<i>Vibrio sp.</i> TH21_20A_OC7	unknown	Oyster, Thau lagoon, France	20°C	eptA-, mcr-; [Col-R]	this study	ERR12116511	76	5,4	4790	68X

<i>Vibrio sp.</i> TH21_20A_OB7	Harveyi	Oyster, Thau lagoon, France	20°C	eptA-1; [Col-R]	this study	ERR12116517	177	6	5667	8X
<i>Vibrio jasicida</i> TH21_20A_OE8	Harveyi	Oyster, Thau lagoon, France	20°C	eptA-1; [Col-R]	this study	ERR12116518	215	6,3	5824	33X
<i>Vibrio owensii</i> TH21_37_OE7	Harveyi	Oyster, Thau lagoon, France	37°C	eptA-1; [Col-R]	this study	ERR12116513	71	5,9	5438	47X
<i>Vibrio alginolyticus</i> TH21_37_OE9	Harveyi	Oyster, Thau lagoon, France	37°C	eptA-3; [Col-R]	this study	ERR12116514	68	5,3	4893	54X
<i>Vibrio alginolyticus</i> TH21_37A_OE10	Harveyi	Oyster, Thau lagoon, France	37°C	eptA-3; [Col-R]	this study	ERR12116515	75	5,1	4690	53X
<i>Vibrio alginolyticus</i> TH21_37A_OE12	Harveyi	Oyster, Thau lagoon, France	37°C	eptA-3; [Col-R]	this study	ERR12116516	88	5,3	4877	25X
<i>Vibrio harveyi</i> TH15_F5_F11 $\Delta rstA$	Harveyi	na	20°C	<i>DrstA</i>	this study					
<i>Vibrio harveyi</i> TH15_F5_F11 $\Delta rstA$ pMRB-P _{LAC} -rstA	Harveyi	na	20°C	$\Delta rstA$, <i>rstA</i> under plac promoter in pMRB plasmid	this study					
<i>Vibrio harveyi</i> TH15_F5_F11 $\Delta rstA$ pMRB-P _{LAC} -gfp	Harveyi	na	20°C	$\Delta rstA$, <i>gfp</i> under plac promoter in pMRB plasmid	this study					
<i>E. coli</i> P3813		na	37°C	<i>lacIQ</i> , <i>thi1</i> , <i>supE44</i> , <i>endA1</i> , <i>recA1</i> , <i>hsdR17</i> , <i>gyrA462</i> , <i>zei298::Tn10</i> , <i>DthyA::(erm- pir116)</i> [Tc ^R Erm ^R]	(Le Roux et al., 2007)					
<i>E. coli</i> b3914		na	37°C	(F ⁻) RP4-2- Tc::Mu DdapA ::(<i>erm- pir116</i>), <i>gyrA462</i> ,	(Le Roux et al., 2007)					

				<i>zei298::Tn10</i> [Km ^R Em ^R Tc ^R]	
<i>E. coli</i> TOP10		na	37°C	TOP10 Chemically Competent <i>E.</i> <i>coli</i>	Invitrogen
<i>Vibrio</i> <i>parahaemolyticus</i> IFVp22	Harveyi	Mussel, English channel, France	37°C	eptA; [Col-R]	(Sorée M. et al., 2024)
<i>Vibrio vulnificus</i> CECT4999	Vulnificus	Eel, Spain	37°C	eptA; [Col-R]	(Murciano C. et al., 2017)

Plasmids	Description			Reference
pSW7848T	<i>oriV_{R6K}</i> ; <i>oriT_{RP4}</i> ; <i>araC</i> -P _{BADccdB} ; [CmR]			(Val et al., 2012)
pSW7848 Δ <i>rstA</i>	pSW7848T :: Δ <i>rstA</i>			this study
pMRB-P _{LAC} - <i>gfp</i>	<i>oriVR6Kg</i> ; <i>oriTRP4</i> ; <i>oriVpB1067</i> ; P _{LAC} - <i>gfp</i> [CmR]			(Le Roux et al., 2011)
pMRB-P _{LAC} - <i>rstA</i>	<i>oriVR6Kg</i> ; <i>oriTRP4</i> ; <i>oriVpB1067</i> P _{LAC} - <i>rstA</i> [CmR]			this study
pBAD-TOPO	pBAD TOPO® TA Expression Kit (Catalog nos. K4300-01, K4300-40)			Invitrogen
pBAD-TOPO- <i>dgkA-eptA-1</i>	<i>dgkA-eptA-1</i> under pBAD promoter in pBAD TOPO® TA			this study
pBAD-TOPO- <i>eptA-1</i>	<i>eptA-1</i> under pBAD promoter in pBAD TOPO® TA			this study
pBAD-TOPO- <i>dgkA</i>	<i>dgkA</i> under pBAD promoter in pBAD TOPO® TA			this study
pBAD-TOPO- <i>eptA-4</i>	<i>eptA-4</i> under pBAD promoter in pBAD TOPO® TA			this study

Table S5. List of primers used in this study

Name	Sequence 5'-3'	Description	DNA matrix
240423-1	GTATCGATAAGCTTGATATCGAATTCCCTTCTCTAATGCCCTTACG	<i>rstA</i> gene deletion-1	<i>Vibrio harveyi</i> Th15_F5_F11
240423-2	GACGATCCTAAACTGCAAGCCCCTGATGCTTGGAATGCGTAATG	<i>rstA</i> gene deletion-2	<i>Vibrio harveyi</i> Th15_F5_F11
240423-3	CATTACGCATTCCAAGCATCAGGGGCTTGCAGTTTAGGATCGTC	<i>rstA</i> gene deletion-3	<i>Vibrio harveyi</i> Th15_F5_F11
240423-4	CCCCGGGCTGCAGGAATTCTGTTTCGTCTGTTCTGGTGC	<i>rstA</i> gene deletion-4	<i>Vibrio harveyi</i> Th15_F5_F11
120523-1	GTGAGCGGATAACAAAGGAAGGGCCCATGAATGTACAAGACACTAAGC	<i>rstA</i> complementation (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
120523-2	CGACGCGTCTGCAGCTCGAGTTACGCATTCCAAGCATCAG	<i>rstA</i> complementation (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11
240423-10	AGTGCACCTGCGCCAGCACC	PCR <i>rstA</i> (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
240423-11	AGGCATCGCAACTAAACAGC	PCR <i>rstA</i> (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11
V1-dgkA-eptA-F	CACCAGGAGATAATAATTGAAACCAGGTAAAAACAGG	<i>dgkA</i> cloning (forward)	<i>Vibrio owensii</i> Th15_Z_G08
V1-dgkA-R	CTAAAGTAAAATCGACGCCCCAAACAAATAGGG	<i>dgkA</i> cloning (reverse)	<i>Vibrio owensii</i> Th15_Z_G08
V1-eptA-F	CACCAGGAGATAATAAATGAAGACAATTGAACTCCC	<i>eptA</i> -1 cloning (forward)	<i>Vibrio owensii</i> Th15_Z_G08
V1-dgkA-eptA-R	TTAGCTTTGACGACAAGTCTTAAAGATATCTAAAGCG	<i>eptA</i> -1 cloning (reverse)	<i>Vibrio owensii</i> Th15_Z_G08
V4-eptA-F	CACCAGGAGATAATAAATGAATCTACCACGCTTAAAC	<i>eptA</i> -4 cloning (forward)	<i>Vibrio splendidus</i> 7T7-2
V4-eptA-R	CTACCGACACGCAGCAAAAATGTC	<i>eptA</i> -4 cloning (reverse)	<i>Vibrio splendidus</i> 7T7-3
dgkA_32F	GGCTCAGCAGTATAGGTCCC	<i>dgkA</i> RT-qPCR (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
dgkA_94R	CGTCCAATATCCGGCGAATG	<i>dgkA</i> RT-qPCR (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11
eptA_175F	TTTGCAGCGCTGAACTTCTT	<i>eptA</i> -1 RT-qPCR (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
eptA_296R	AGTGTGCCGTAGTTGTAGCT	<i>eptA</i> -1 RT-qPCR (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11
VH_2913_640F	AAGAAGCACGCAATCATCGC	VH_2913 RT-qPCR (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
VH_2913_750R	GTGACCAAGAACCGTTGCAC	VH_2913 RT-qPCR (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11
VH_0852_53F	CCCATGCAGCGATTGAAGTG	VH_0852 RT-qPCR (forward)	<i>Vibrio harveyi</i> Th15_F5_F11
VH_0852_185R	TGAGCCAATTCTGCATTCTGA	VH_0852 RT-qPCR (reverse)	<i>Vibrio harveyi</i> Th15_F5_F11