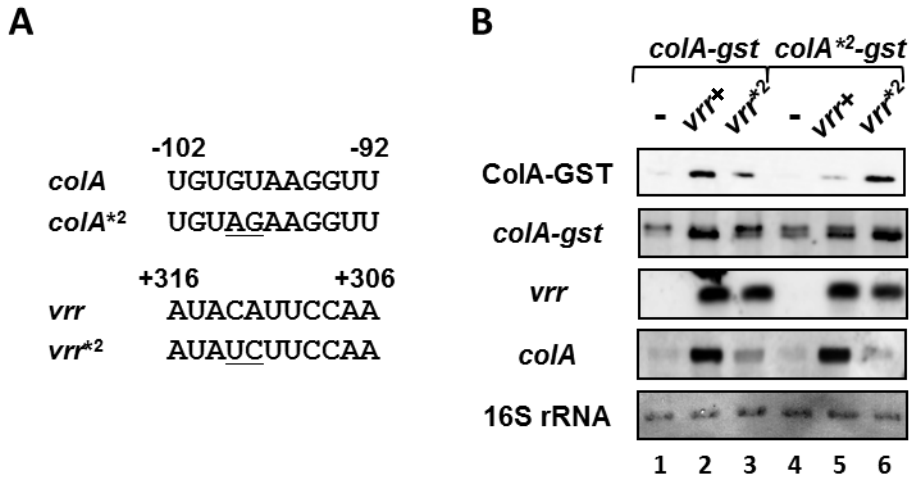
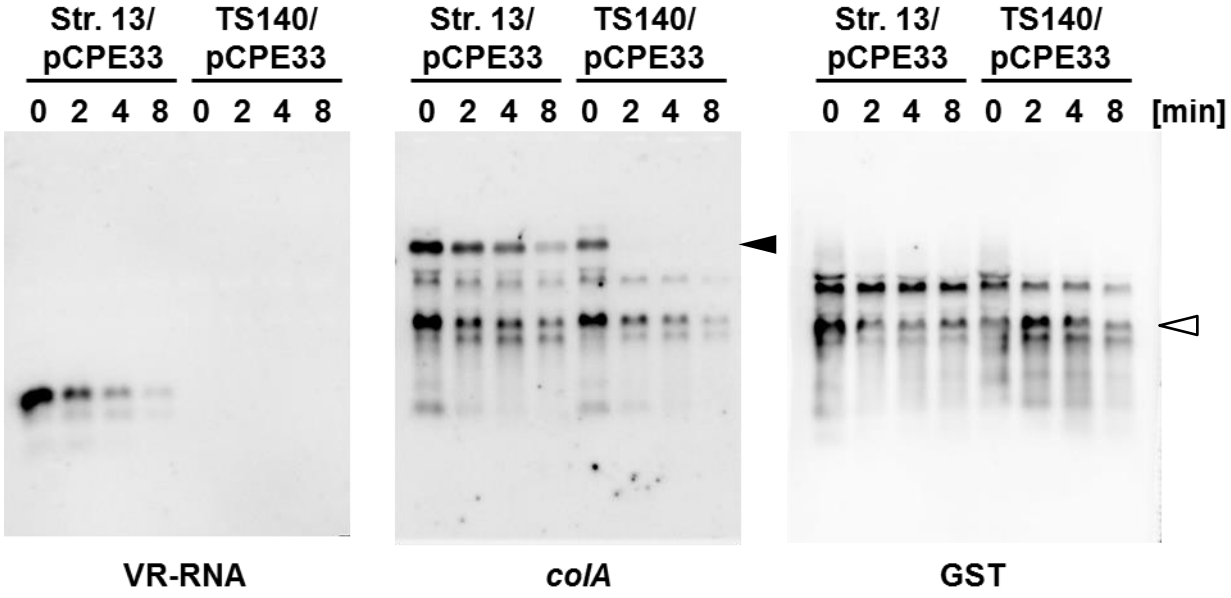
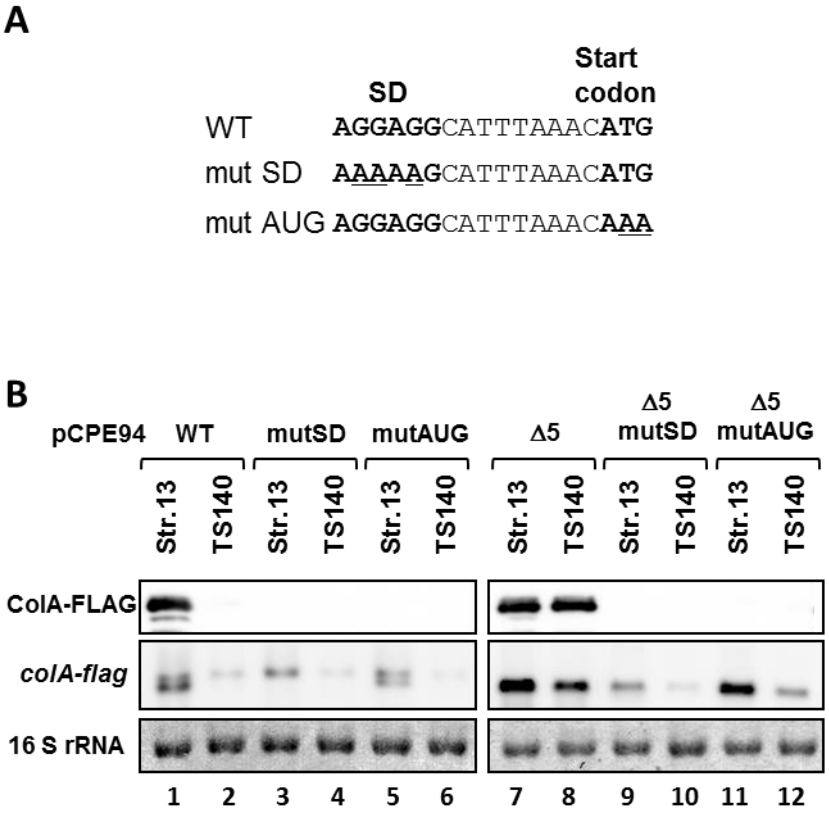


Supplementary Figure S1 Complementarity between VR-RNA and *colA* mRNA 5'UTR is important for *colA* regulation.

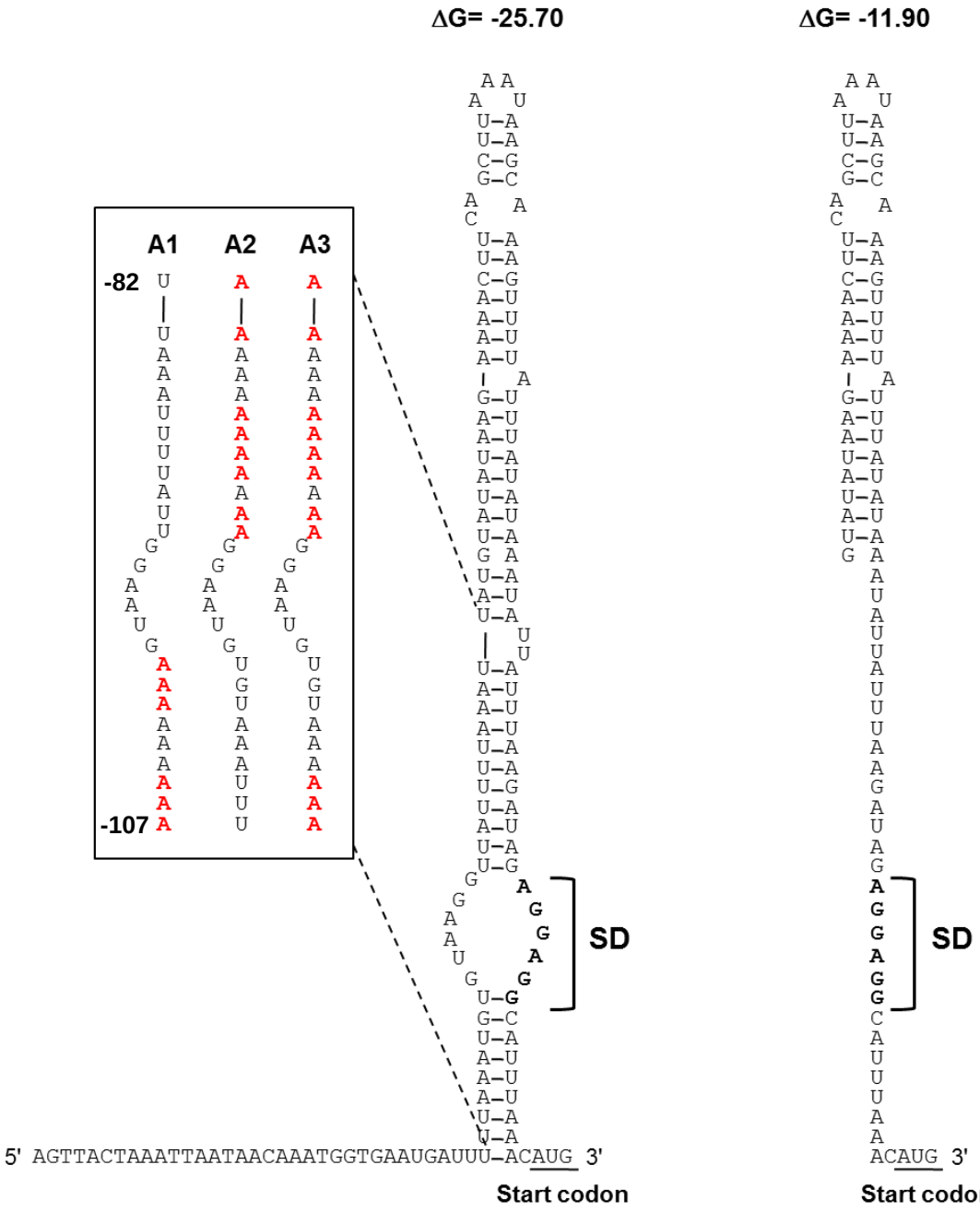
(A) Mutation sites within VR-RNA-*colA* RNA duplex. Mutation sites in *colA* mRNA 5'UTR or VR-RNA are underlined. (B) Western and Northern blots of VR-RNA-deficient strains carrying *colA-gst* or *colA-gst* mut2 and *vrr*, a gene encoding VR-RNA, or *vrr* mut2 co-expression vector. Each lane was loaded with 0.02 A_{280} units of extracellular protein or with 0.5 μg of total RNA from cells grown to the mid-exponential growth phase at 37°C. GST fusion protein was probed with anti-GST antibody (top panel). Chromosomally encoded *colA* mRNA, *colA-gst* and plasmid-encoded VR-RNA were detected using specific probes. Methylene blue-stained 16S rRNAs are indicated at bottom as loading controls.



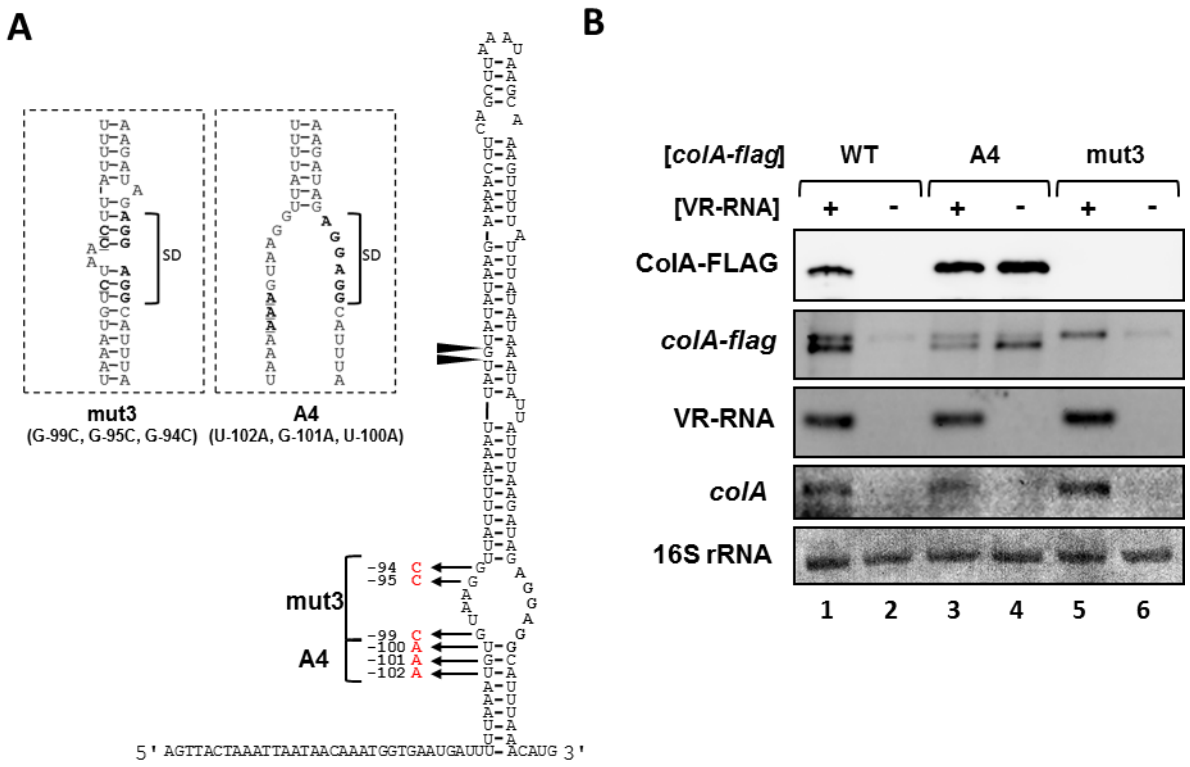
Supplementary Figure S2 Stability of *colA-gst* mRNA. Northern blot analysis was performed using 0.5 μg of total RNA isolated from *C. perfringens* strain 13 and TS140 (*vrr*-deficient mutant) harboring pCPE33 containing *colA-gst* gene. These strains were grown to the mid-exponential phase at 37°C in GAM broth and then, rifampicin (200 μg/ml) was added to the culture. Total RNA (1 μg) isolated from the cells harvested at indicated time after addition of rifampicin were loaded in each lane. VR-RNA, *colA* and *gst* gene-specific probes were used for detection of VR-RNA, chromosomally encoded *colA* and *colA-gst* mRNA, respectively. Filled and open triangles represent *colA* and *colA-gst* transcripts, respectively.



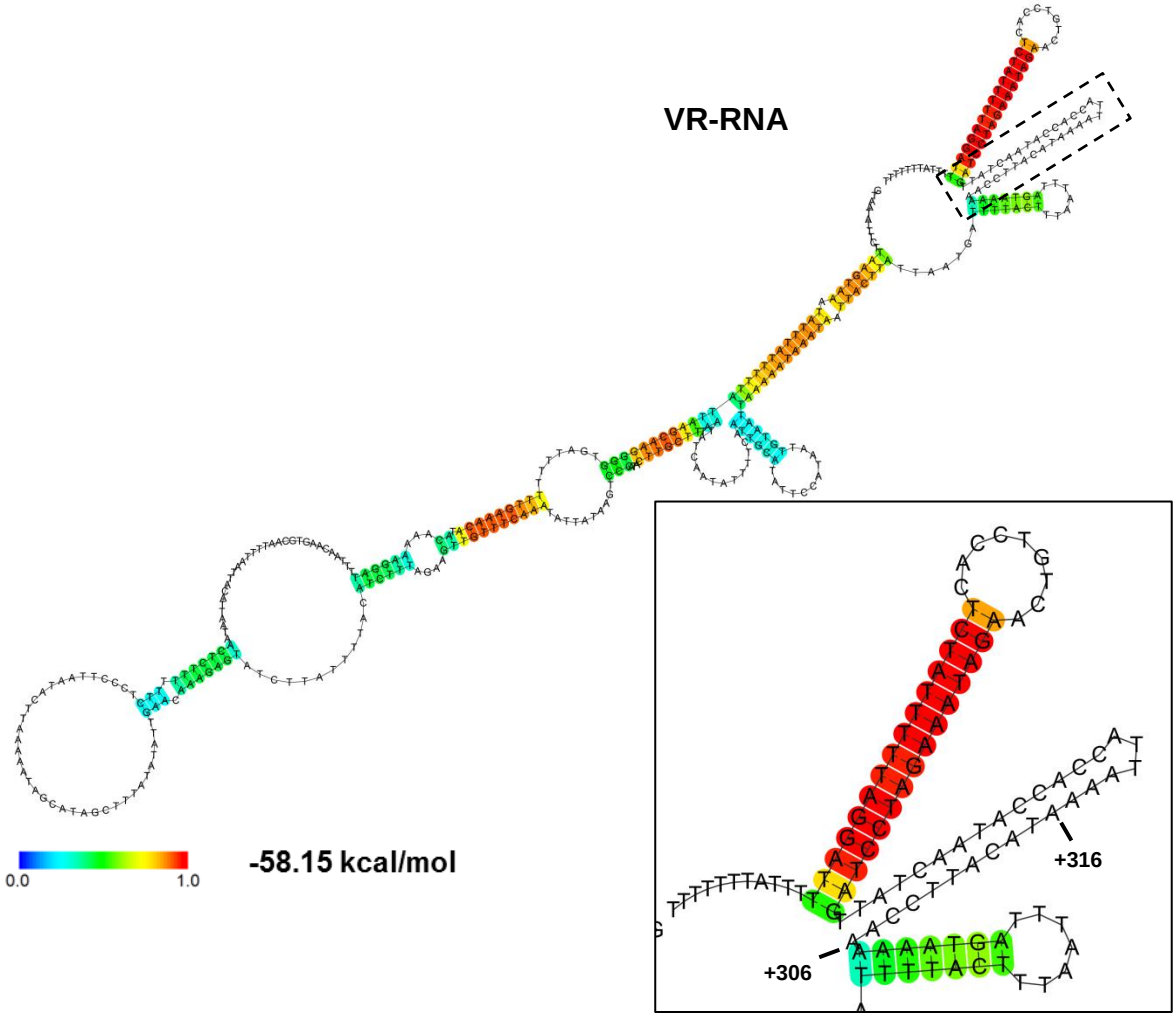
Supplementary Figure S3 Ribosome binding to the SD sequence of *colA-flag* mRNA is requred to stabilization of the transcript. A. Ribosome binding site and start codon of the *colA-flag* gene. The mutated nucleotides in the mutant *colA-flag* gene are indicated by red. Western and northern blot analysis is carryed out using 0.2 $O.D._{600}$ unit of extracellular proten and 1 μg of total RNA isolated from *C. perfringenes* strain 13 and TS140 harboring pCPE94 vector in which the SD sequence or AUG start codon of *colA-flag* gene was mutated. The processed transcript was initialry transcribed from pCPE94Δ5 derivative in which processed region in *colA-flag* 5'UTR was removed. ColA-FLAG protein and *colA-flag* mRNA were detected by using anti-DYKDDDK antibody and DIG-labeled *colA* gene-specific probe. Methylene blue-stained 16S rRNAs are indicated at bottom as loading controls.



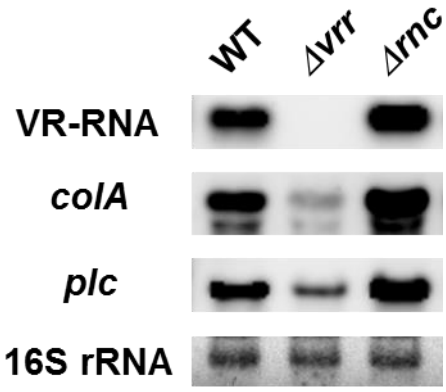
Supplementary Figure S4 Secondary structure of *colA* mRNA and the region into which the mutations were introduced. The secondary structure prediction of primary and processed *colA* transcripts are represented on the right and left side, respectively. SD sequence and start codon were indicated. The inset shows the mutated bases which are indicated by red fonts.



Supplementary Figure S5 Disruption and strengthening of stem-loop structure in *colA* mRNA induce and inhibit the translation, respectively. (A) The location of the point mutations which disrupt or strengthen the stem-loop structure is indicated on the predicted *colA* mRNA secondary structure. (B) ColA-FLAG protein and *colA-flag* mRNA expression was analyzed on western and northern blots. Extracellular protein (0.02 $O.D._{600}$ unit) or Total RNA (1 μg) were extracted from *C. perfringens* strain 13 and TS140 harboring various *colA-flag* genes grown to the mid-exponential phase, and were probed with anti-DYKDDDK antibody or DIG-labeled *colA* and *vrr* gene-specific probes. Methylene blue-stained 16S rRNAs are indicated at bottom as loading controls.



Supplementary Figure S6 Secondary structure of VR-RNA. The RNA secondary structure was predicted by CentroidFold (http://www.ncrna.org/centroidfold/). Each predicted base pairing is indicated by the heat color gradation from blue to red corresponding to the base pairing probability from 0 to 1. The region important for base pairing with *colA* mRNA is boxed by dashed line and is uncolored, indicating that RNAs within the region are unpaired. The right inset shows a larger magnification of 3' region of VR-RNA.



Supplementary Figure S7 Ribonuclease III is not involved in *colA* expression. VR-RNA and *colA* and *plc* mRNA expression was analyzed by northern blot analysis using DIG-labeled *vrr*, *colA* and *plc* gene-specific probes. Total RNA (2 μg) extracted from strain 13 and *vrr* or *rnc*-deficient strains grown to the mid-exponential phase were used in each lane. Methylene blue-stained 16S rRNAs are indicated at bottom as loading controls.

Table S1. Oligonucleotides used in this study

Name	Sequence 5' to 3'	Used for
NOB-0390	GGACTAGTCCAATTCAAATTATTCTCG	VR-RNA-expression vector
NOB-0393	GCGCGCCGGCAAAAATAATTATCATTAGT	VR-RNA-expression vector
NOB-0068	GGCCGGATCCTTACTGAGTTGCCAATAAAG	*colA-flag*-expression vector
NOB-0084	GCGCGTCGACTTAACCTTTATCGTCATCATCTTTATAATCGTTTTTAGCATCATATCCCTTAG	*colA-flag*-expression vector
NOB-0398	GGTTTTTTACTATTAATAAGTAAAATCATT	VR-RNA mut5 mutation
NOB-0399	TTACATAAAATTACCACCATAACTATTGAT	VR-RNA mut5 mutation
NOB-0400	TAATTTAGTAAAAAACGTTACATAAAATTACCACC	VR-RNA mut1 mutation
NOB-0401	GGTAATTTTATGTAACGTTTTTTACTAAATTAAAG	VR-RNA mut1 mutation
NOB-0402	TAATTTAGTAAAAAACGTTCTATAAAATTACCACC	VR-RNA mut2 mutation
NOB-0403	GGTAATTTTATAGAACGTTTTTTACTAAATTAAAG	VR-RNA mut2 mutation
NOB-0388	ACATAAAATTACCAgCATAACTATTGATCC	VR-RNA mut4 mutation
NOB-0389	GATCAATAGTTATGcTGGTAATTTTATGTA	VR-RNA mut4 mutation
NOB-0391	TAATTTAGTAAAAAAGGTTAGATAAAATTACCACC	VR-RNA mut3 mutation
NOB-0392	GGTAATTTTATCTAACCTTTTTTACTAAATTAAAG	VR-RNA mut3 mutation
NOB-0383	GGCCGAATTCGTAATATTTCTTAAGTAAA	*in vitro* transcription vector
NOB-0386	GCGCAAGCTTAATCCTAAAAATAGAGTGGACAG	*in vitro* transcription vector
NOB-0011	GCCGGGATCCGTTTAAATGCCTCCTCTATC	*in vitro* transcription vector
NOB-0370	CTAATACGACTCACTATAGGGAGTTACTAAATTAATAACAAATGGTG	*in vitro* transcription vector
NOB-0045	TGAATGATTTTTAAAaaaGTAAGGTTATTTTAAA	*colA* A4 mutation
NOB-0046	TTTAAAATAACCTTACtttTTTAAAAATCATTCA	*colA* A4 mutation
NOB-0047	GGAGGCATTTAAACAAAAAGAAAAACTTAAAAAG	*colA* AUG mutation
NOB-0048	CTTTTTAAGTTTTTCTTTTTGTTTAAATGCCTCC	*colA* AUG mutation
NOB-0051	TGAATGATTTTTAAATaTGTAAGGTTATTTTAAA	*colA* G-101A mutation
NOB-0052	TTTAAAATAACCTTACAtATTTAAAAATCATTCA	*colA* G-101A mutation
NOB-0053	TGATTTTTAAATGTCTAACCTTATTTTAAATTATG	*colA* mut3 mutation
NOB-0054	AATTTAAAATAAGGTTAGACATTTAAAAATCATTC	*colA* mut3 mutation
NOB-0055	TGATTTTTAAATGTGTAACGTTATTTTAAATTATG	*colA* mut1 mutation
NOB-0056	AATTTAAAATAACGTTACACATTTAAAAATCATTC	*colA* mut1 mutation
NOB-0057	GAATGATTTTTAAATGTAGAACGTTATTTTAAATTATG	*colA* mut2 mutation
NOB-0058	AATTTAAAATAACGTTCTACATTTAAAAATCATTC	*colA* mut2 mutation
NOB-0059	CAAATGGTGAATGATTaaaAAAaaaGTAAGGTTATTTTAAATTATG	*colA* A1 mutation
NOB-0060	TTTAAAATAACCTTACtttTTTtttAATCATTCACCATTTG	*colA* A1 mutation
NOB-0063	ATGTGTAAGGTTAaaaaAAAaaATGTATATAAGAAA	*colA* A2 mutation
NOB-0064	TTCTTATATACATttTTTttttTAACCTTACACATT	*colA* A2 mutation
NOB-0065	TAACCTTACACATTTtttAATCATTCACCATTTG	*colA* A3mutation
NOB-0107	TTTAAATTATtTATATAAGAAAACTTCAGC	*colA* G-79U mutation
NOB-0108	AGTTTTCTTATATAaATAATTTAAAATAACCTTAC	*colA* G-79U mutation
NOB-0109	TTTAAATTATaTATATAAGAAAACTTCAGC	*colA* G-79A mutation
NOB-0110	AGTTTTCTTATATAtATAATTTAAAATAACCTTAC	*colA* G-79A mutation
NOB-0111	TTTAAATTATcTATATAAGAAAACTTCAGC	*colA* G-79C mutation
NOB-0112	AGTTTTCTTATATAgATAATTTAAAATAACCTTAC	*colA* G-79C mutation

Table S2. Plasmids used in this study

Name	Description	Refference
pJIR418	*E. coli-C. perfringens* shuttle vector, Cm^R, Em^R	Sloan J *et al.* (1992)
pGEM3zf (+)	cloning vector, AmpR	promega
pVrr	VR-RNA-expression vector	This study
pVrrmut1	VR-RNA mut1-expression vector	This study
pVrrmut2	VR-RNA mut2-expression vector	This study
pVrrmut3	VR-RNA mut3-expression vector	This study
pVrrmut4	VR-RNA mut4-expression vector	This study
pVrrmut5	VR-RNA mut5-expression vector	This study
pVrrmut6	VR-RNA mut6-expression vector	This study
pCPE33	*colA-gst*-expression vector	Obana *et al.* (2010)
pCPE31	*colA* (D+2-78)-*gst-expression* vector	Obana *et al.* (2010)
pCPE111	*colA-gst* and VR-RNA-expression vector	This study
pCPE112	*colA-gst* and VR-RNA mut1-expression vector	This study
pCPE113	*colA mut1-gst* and VR-RNA-expression vector	This study
pCPE114	*colA mut1-gst* and VR-RNA mut1-expression vector	This study
pCPE210	*colA-gst mut2*-expression vector	This study
pCPE212	*colA-gst* and VR-RNA mut2-expression vector	This study
pCPE213	*colA mut2-gst* and VR-RNA-expression vector	This study
pCPE214	*colA mut2-gst* and VR-RNA mut2-expression vector	This study
pCPE310	*colA-gst mut3*-expression vector	This study
pCPE312	*colA-gst* and VR-RNA mut3-expression vector	This study
pCPE313	*colA mut3-gst* and VR-RNA-expression vector	This study
pCPE314	*colA mut3-gst* and VR-RNA mut3-expression vector	This study
pCPE94	*colA-flag*-expression vector	This study
pCPE94Δ5	*colA* (D+2-78)-*flag-expression* vector	This study
pCPE94A1	*colA-flag* A1-expression vector	This study
pCPE94A2	*colA-flag* A2-expression vector	This study
pCPE94A3	*colA-flag* A3-expression vector	This study
pCPE94A4	*colA-flag* A4-expression vector	This study
pCPE94A5	*colA* (G-101A)-*flag*-expression vector	This study
pCPE94GC3	*colA-flag* GC3-expression vector	This study
pCPE95	*colA* (G-79U)-*flag*-expression vector	This study
pCPE96	*colA* (G-79A)-*flag*-expression vector	This study
pCPE97	*colA* (G-79C)-*flag*-expression vector	This study
pCPE98	*colA-flag* with mutated SD-expression vector	This study
pCPE99	*colA-flag* with mutated AUG start codon-expression vector	This study
pCPE95-1	*colA* (G-79U, U-74G)-*flag*-expression vector	This study
pCPE95-2	*colA* (G-79U, U-80G)-*flag*-expression vector	This study
pNOE40	T7 template of VR-RNA derived from pGEM3zf (+)	This study
pNOE41	T7 template of VR-RNA mut1 derived from pGEM3zf (+)	This study
pNOE42	T7 template of VR-RNA mut2 derived from pGEM3zf (+)	This study
pNOE43	T7 template of VR-RNA mut3 derived from pGEM3zf (+)	This study
pNOE44	T7 template of VR-RNA mut4 derived from pGEM3zf (+)	This study
pNOE45	T7 template of VR-RNA mut5 derived from pGEM3zf (+)	This study
pNOE46	T7 template of VR-RNA mut6 derived from pGEM3zf (+)	This study