Supplementary Tables

Supplementary Table 1: Bacterial Strains and Plasmids Used in This Study

Name	Relevant Features	Source/Reference
Bacterial strains:		
Escherichia coli		
DH5α	F$^-$ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^- m_K^+$), λ$^-$	Laboratory collection
MG1655		
Top10	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ *lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	ThermoFisher
RFP	Top10 (pTOPO::rfp), strain 8186	Laboratory collection
XL2blue	*endA1 gyrA96*(nal^R) *thi*-1 *recA1 relA1* lac *glnV*44 F'[::Tn10 proAB$^+$ lacI^q Δ(lacZ)M15 Amy Cm^R] hsdR17(r_K^- m_K^+)	Stratagene
SXT	HW220 (CAG18439 *pfrC*::SXT)	V. Burrus
β3914	*β2163* [(F$^-$) RP4-2-Tc::Mu *ΔdapA*::(erm-*pir*116)*] gyrA*462 zei-298::Tn10	(1)
Π1	DH5α *ΔthyA::(erm- pir*116)	(2)
Vibrio cholerae		
Vibrio cholerae O1	*Vibrio cholerae* serotype O1 biotype El Tor strain N19691 (contains a frame shift into *lacZ* gene)	(3)
ΔlacZ	*Vibrio cholerae O1- ΔlacZ*	(4)
ΔtoxRS	*Vibrio cholerae O1- ΔtoxRS*	This study
O1-GFP	*Vibrio cholerae O1-* constitutive expression of *gfp*	Laboratory collection
O139	MO10 toxigenic 1992 clinical isolate from India, SXT^{MO10+}, Su^{R}Tm^{R}Cm^{R}Sm^R	(5)
Other strains		
Vibrio vulnificus	WT	Laboratory collection
Vibrio mimicus	WT	Laboratory collection
Salmonella typhimurium	WT	J.M. Ghigo
Citrobacter rodentium	WT	J.M. Ghigo
Plasmids:		
pBAD43	ori pSC101, Sp^R	(6)
pBAD30	ori pACYC184, Carb^R	(6)
pBAD24	ori pBR322, Carb^R	(6)
pSU38	ori p15A, Kan^R	(7)
pSW7848	suicide plasmid for allele exchange – *ori*V_{R6K}, *ori*T_{RP4} *araC*-P_{BAD}-*ccdB*	(4)
pRFP	*rfp* gene cloned in pTOPO	Laboratory collection

Supplementary Table 1: (continuation)

Name	Relevant Features	Source/Reference
pTox	pBAD43-ccdB (between EcoRI-XbaI)	This study
pToxInt	pBAD43-ccdB/intein operon (between EcoRI-XbaI)	This study
pN	pBAD43-ccdB/intein N-terminal fusion (between EcoRI-XbaI)	This study
pCcdB-Int C	pSU38-ccdB/intein C-terminal fusion (between EcoRI-XbaI)	This study
pParE2-Int N	pBAD43-parE2/intein N-terminal fusion (between EcoRI-XbaI)	This study
pParE2-Int C	pSU38-parE2/intein C-terminal fusion (between EcoRI-XbaI)	This study
pHigB2-Int N	pBAD43-higB2/intein N-terminal fusion (between EcoRI-XbaI)	This study
pHigB2-Int C	pSU38-higB2/intein C-terminal fusion (between EcoRI-XbaI)	This study
pRelE4-Int N-1	pBAD43-relE4/intein N-terminal-1 fusion (between EcoRI-XbaI)	This study
pRelE4-Int C-1	pSU38-relE4/intein C-terminal-1 fusion (between EcoRI-XbaI)	This study
pRelE4-Int N-2	pBAD43-relE4/intein N-terminal-2 fusion (between EcoRI-XbaI)	This study
pRelE4-Int C-2	pSU38-RelE4/intein C-terminal-2 fusion (between EcoRI-XbaI)	This study
pCcdB-Int n*	pN with a mutation that blocks intein splicing	This study
pParE2-Int n*	pParE2-Int N with a mutation that blocks intein splicing	This study
pHigB2-Int n*	pHigB2-Int N with a mutation that blocks intein splicing	This study
pRelE4-Int n*-1	pRelE4-Int N-1 with a mutation that blocks intein splicing	This study
pRelE4-Int n*-2	pRelE4-Int N-2 with a mutation that blocks intein splicing	This study
pU-BAD	pBAD43-ccdB/int N-fusion (*ompU-1* promoter), C-fusion under P_{BAD}	This study
pRS	*toxRS* operon from *V. cholerae* cloned into pBAD30 (SacI-XbaI) keeping natural RBS sequence of *toxR*	This study
pN_{ctrl}	ori pSC101, Sp^R, ccdB-Int N-terminal fusion (P_{ompU}), $oriT_{RP4}$	This study
$ptox_{ctrl}$	ori pSC101, Sp^R, ccdB-Int N- and C-fusion (operon- P_{BAD}), $oriT_{RP4}$	This study
pPW	ori pSC101, Sp^R, ccdB-Int N- and C-fusion (operon- *ompU*-1 promoter) $oriT_{RP4}$	This study
ptoxRS-UP-DOW	pSW7848-500bp upstream and downstream of *toxRS* operon	This study
p*ccdA*	ori pBR322, $Carb^R$, ccdA antitoxin (P_{BAD})	This study
pPW(RBS)	pPW with canonical RBS sequence	This study
pPLA	pCcdBInt, *ccdA* (PL promoter)	This study
pABRW	pCcdBInt, *ccdA* (PL promoter), $oriT_{RP4}$	This study
pFW	pFW1, canonical RBS in ompU-1 promoter, extra operator O1-sequence into PL promoter	This study
pPW-R6K	pPW with an R6K replication origin	This study
pN_{ctrl}-R6K	pN_{ctrl} with an R6K replication origin	This study
pFW-R6K	pDW with an R6K replication origin	This study

Supplementary Table 2. Conjugation efficiency in the different conditions and strains used. Conjugation efficiency is calculated as the number of transconjugants divided by the total bacteria and multiplied by 100 to obtain the percentage (%). We have also analyzed the conjugation of the pFW plasmid in MG1655 bacteria using a donor:recipient ratio of 1:1 and obtaining more than 70% of conjugation efficiency.

Donor	Recipient	Figure	Donor::recipient, conditions	Total CFU/ml	Transconjugants (CFU/ml)	Efficiency (%)
β 3914-pN$_{control}$	*V. cholerae* O139	-	1:1, without DAP	$3.4 \cdot 10^8$	$2.4 \cdot 10^7$	7 %
β 3914-pN$_{control}$	*V. cholerae* O139	-	1:1, with DAP	$2.9 \cdot 10^8$	$2.7 \cdot 10^7$	9.3 %
β 3914-pN$_{control}$	*V. cholerae* O139	-	10:1, water	$1.8 \cdot 10^6$	$5.6 \cdot 10^2$	0.03 %
β 3914-pN$_{control}$	*V. cholerae* O1	-	1:1	$1.3 \cdot 10^8$	$8.2 \cdot 10^6$	6.3 %
β 3914-pN$_{control}$	*V. cholerae* O1	-	2:1	$1.3 \cdot 10^8$	$5.4 \cdot 10^6$	4.2 %
β 3914-pN$_{control}$	*V. cholerae* O1	-	3:1	$1.2 \cdot 10^8$	$3.8 \cdot 10^6$	3.16 %
β 3914-pN$_{control}$	*V. cholerae* O139	-	1:1	$1.8 \cdot 10^8$	$7.8 \cdot 10^6$	4.3 %
β 3914-pN$_{control}$	*V. cholerae* O139	-	2:1	$1.3 \cdot 10^8$	$8.1 \cdot 10^6$	6.2 %
β 3914-pN$_{control}$	*V. cholerae* O139	-	(3:1)	$0.9 \cdot 10^8$	$8.5 \cdot 10^6$	9.4%
β 3914-pN$_{control}$	*V. cholerae* O139	Fig. 4a	1000:1, zebrafish	$8 \cdot 10^5$	57.5	0.0071 %
β 3914-pN$_{control}$	*V. cholerae* O139+O1	Fig. 4a	10:1, zebrafish	$7 \cdot 10^5$	200	0.025 %
β 3914-pN$_{control}$	*V. cholerae* O139	Fig. 4b	1:1, *Artemia*	$16.5 \cdot 10^6$	2170	0.013 %
β 3914-pN$_{control}$	*V. cholerae* O139+O1	Fig. 4b	1:1, *Artemia*	$16.7 \cdot 10^6$	3465	0.020 %
β 3914-pFW	MG1655	-	1:1	$20 \cdot 10^6$	$14.7 \cdot 10^6$	72.26 %
β 3914-pABRW	MG1655	-	10:1	$3.2 \cdot 10^6$	$2.8 \cdot 10^6$	89.58 %

Supplementary Table 3. Escape mutants obtained after the conjugation of the genetic weapon plasmids in this study. We performed conjugation with the weapon plasmids in the targeted bacteria. After conjugation, bacteria were spread in the presence of spectinomycin in order to detect transconjugants that are able to survive after the acquisition of the plasmid weapon. Numbers on the table indicate the CFU/ml of transconjugants that are named: escape mutants, for each weapon plasmid/strain in comparison with the total bacteria after conjugation.

	Weapons		Plasmids	
Strains	pPW	$pPW_{(RBS)}$	pABRW	pFW
V. cholerae O1	$6\text{-}log_{10}$ (n=4)	$7\text{-}8\text{-}log_{10}$ (n=6)	-	-
V. cholerae O139	$6\text{-}log_{10}$ (n=4)	$7\text{-}8\text{-}log_{10}$ (n=6)	$6\text{-}log_{10}$ (n=5)	$6\text{-}log_{10}$ (n=7)
E. coli - SXT	-	-	$5\text{-}log_{10}$ (n=7)	-

Supplementary Table 4. Escape mutants analyzed in this study. We analyzed by PCR the *V. cholerae* escape mutants that survived plasmid acquisition through conjugation. Plasmids used are indicated in parentheses and numbers indicate the number of analyzed clones. The inactivating insertion sequence identified by sequencing as *V. cholerae* DNA 116-17a plasmid seq. ID: LN831185. PCR products which size corresponds with the control fragment (toxin-intein amplification) were sent for sequencing and point mutations avoiding the correct translation of the toxin were detected for pPW clones. However for pFW clones, no mutations were detected after sequencing. We then transformed *V. cholerae* serogroups O1 and O139 with these plasmids that looks as pFW. The result showed that transformants were only obtained in O1 serogroup. Then these plasmids are still toxic for *V. cholerae* O139 and fully able to be transferred in O1 serogroup. In order to know why these V. cholerae O139 clones are able to tolerate the conjugation of pFW we performed PCR to analyze the presence of *toxRS* and *setR*. After this analysis *toxRS* was correctly amplified but not *setR*. Then we demonstrated that they have lost the SXT element (see suppl. Table 4).

Clones	*V. cholerae* O1 (pPW)	*V. cholerae* O139 (pPW)	*V. cholerae* O139 (pFW)	Frequency (%)
Correct toxin amplification	5	6	11	47.8 %
Vibrio's Insertion Sequence	6	10	5	46.67 %
No amplification	3	0	0	6.67 %
Total	14	16	16	100 %

Supplementary Table 5. Escape mutants that lost SXT element. Number indicated clones analyzed in MH media containing trimethoprim, which is one of the antibiotic resistant, genes encoded into SXT element.

Clones	*E. coli* –SXT (pABRW)	Frequency (%)	*V. choerae* O139 (pFW)	Frequency (%)
Containing SXT	8	37 %	4	10 %
Lost of SXT	15	63 %	36	90 %
Total	23	100 %	40	100 %

Supplementary Table 6: Primers used in this study

Name	Sequence (5' → 3')
F-CcdB-EcoRI	GGAATTCATGTCTCAATTTACGCTATATAAAA
R-CcdB-XbaI	GCTCTAGAGCAAGCGTTAAATGCCAGTGATTA
F-Int/dB	TAAGAAATGTTTAAGCTATGAAACGGAAATA
R-dB/Int	CTTAAACATTTCTTATCAAGTAGTTCGATTGG
R-Int-XbaI	GCTCTAGATCAATTCGGCAAATTATCAACC
F-Int-EcoRI	GGAATTCGTAAGGAGGTAACATATGATCAAAATAGCCACACGTAAA
R-Int C/ccdB-2	CTTGGTGCATTGAAACAATTAGAAGCTATGA
F-CcdB-C/Int 2	TTTCAATGCACCAAGTCACCTTTGTCCTAC
F-ParE2-EcoRI	GGAATTCAATGAAACCATTTAATCTTACCGTCGC
R-ParE2-XbaI	GCTCTAGATTATGCGCCGAATATTGGGTTCACATC
F-ParE2/Int-1	TTTCAATATTGGTAAATCATGCGATGAAATCCGAG
R-ParE2/Int-1	CTTAAACAGTCGGGATTTTCCGCTAAAAGCCA
F-Int/ParE2-1	ATCCCGACTGTTTAAGCTATGAAACGGAAATA
R-Int/ParE2-1	TTACCAATATTGAAACAATTAGAAGCTATGAAGC
F-HigB2-EcoRI	GGAATTCGATGAAAAGTGTATTTGTCGAATCAAC
R-HigB2/Int-1	CTTAAACACTTTTCATCGAGAAAGTAATAG
F-HigB2/Int-1	TTTCAATAGGCGTTTCTATTTGCTAACC
R-HigB2-XbaI	GCTCTAGATATCACGATTGCTCATTGCGCCACGCCTCC
F-Int/HigB2-1	GATGAAAAGTGTTTAAGCTATGAAACGGAAATA
R-Int/HigB2-1	GAAACGCCTATTGAAACAATTAGAAGCTATGAAGC
F-RelE4-EcoRI	GGAATTCGATGATTTTCTGGGAAGAAGCATCTCTCAATG
R-RelE4/Int-1	CTTAAACAATCACGCTGAACACCGATTAG
F-RelE4/Int-1	TTTCAATGGCATTAGAGGCAGATTGC
R-RelE4-XbaI	GCTCTAGATCAGTCGTTTGGAAATTTTTGTTTCTGATGTAG
F-Int/RelE4-1	CAGCGTGATTGTTTAAGCTATGAAACGGAAATA
R-Int/RelE4-1	CTAATGCCATTGAAACAATTAGAAGCTATGAAGC
R-RelE4/Int-2	CTTAAACAATCAACCCAATACGAAACAATC
F-RelE4/Int-2	TTTCAATGGTTCTAAAATTCGAATAATGCGTG
F-Int/RelE4-2	GGGTTGATTGTTTAAGCTATGAAACGGAAATA
R-Int/RelE4-2	TAGAACCATTGAAACAATTAGAAGCTATGAAGC
R-Int/dB N mut	CCTTTCCCATTTCTTATCAAGTAGTTCGATTGG
F-Int/dB N mut	TAAGAAATGGGAAAGGTATGAAACGGAAATA
F-Int/E4-1 N mut	GCGTGATTGGGAAAGGTATGAAACGGAAATA
F-Int/E4-2 N mut	GGGTTGATTGGGAAAGGTATGAAACGGAAATA
R-Int/E4-1 N mut	CCTTTCCCAATCACGCTGAACACCGATTAG
R-Int/E4-2 N mut	CCTTTCCCAATCAACCCAATACGAAACAATCATTG
F-Int/B2 N mut	GATGAAAAGTGGGAAAGGTATGAAACGGAAATA
F-Int/E2 N mut	AATCCCGACTGGGAAAGGTATGAAACGGAAATA
R-Int/B2 N mut	CCTTTCCCACTTTTCATCGAGAAAGTAATAG
R-Int/E2 N mut	CCTTTCCCAGTCGGGATTTTCCGCTAAAAGCC
R-BAD43-BAD18	CGCCACAGGCAAGGCGATTAAGTTGGGTAACGCCAGG
F-BAD43-BAD18	CAACTATGGATTCGATAAGCAGCATCGCCTGTTTC
R-BAD18-BAD43	ATCGAATCCATAGTTGCCTGACTCCCCGTCGTGTAG
F-BAD18-BAD43	ATCGCCTTGCCTGTGGCGCCGGTGATGCCGGCCACG
4216	TATCAGGGACTGGAAAATCAGAGGGCAGGAACTGCTG
4217	TCCAGTCCCTGATATAGGCGCCAGCAACCGCACC
R-Int-N/Int-C	ACCTCCTTATCAATTCGGCAAATTATCAACCCGCATC
F-Int-C/Int-N	CCGAATTGATAAGGAGGTAACATATGATCAAAATAGCCACAC

Supplementary Table 6: (continuation)

Name	Sequence (5' → 3')
F-toxR-SacI	GGGAGCTCGTCCTCAAAAGAGATATCGATGAGTCATATTGG
R-toxS-XbaI	GCTCTAGACCTTAAGAATTACTGAACAGTACGGTAGAACCATGAC
R-BAD-PU1	GATTTAGGTTGGTAACGAATCAGACAATTGACGGC
F-PompU-1	TACCAACCTAAATCGGGTCGGGTTAGGGTTGAACCATTT
R-PompU-dB	TTGAGACATAAATTTGATTTTTGTGCGACGTAAGCC
F-dB-PompU	CAAATTTATGTCTCAATTTACGCTATATAAAAAC
F-ccdA-EcoRI	GGAATTCACCATGAGAAATCAATATAATACACAAGCGGTAAAGAAAGC
R-ccdA-XbaI	GCTCTAGATTAAAATACTCGGTATGAATCAGAAAAAAGACCGTG
F-toxRup-p7	CTGCGAGGCTGGCCGGCGTCCGTTATCCGAAATGGTCAACGTATTTTTGTC
R-toxRups	CTCAGTCAGGCGATATCTCTTTTGAGTTGTGTCCTAATCC
F-toxSdow	GAGATATCGCCTGACTGAGCGTAGAATAGGACATAAC
R-toxSdow-p7	CAAGCTTATCGATACCGTCGAGTATGCCGCGAGCTATGGCGTGCTGGAAGGCGATAC
F-BAD-pSW	GGTACCCCGCCGGTGATGCCGGCCACGATG
F-pSW23-BAD	ACCGGCGGGGTACCAGCGCTTTTCCGCTGC
R-BAD43-oriT	CTGGCCGGCGGCTCACTGCCCGCTTTCCAGTCGGG
R-OriT-BAD43	CAGTGAGCCGCCGGCCAGCCTCGCAGAGCA
R-PL-ccdA	GATTTCTCATACCGTCTCCTGTTACAATAATAACTGTTAC
F-PL-plasmid	CTGTTTCAAGCCCGTATTCTACAAATAAAACTGTAGCC
F-ccdA-PL	GACGGTATGAGAAATCAATATAATACACAAGCGGTAAAG
R-ccdA-plasmid	CAGCCTTTAAAATACTCGGTATGAATCAGAAAAAAGACCGTG
F-plasmid-dA	gtattttaaAGGCTGTCTATGTGTGACTGTTGAGC
R-plasmid-PL	gggcttGAAACAGGCGATGCTGCTTATCGAATC
F-ccdB-SD-OK	CACAAAGGAGGTATTCCATGTCTCAATTTACGCTATATAAAAAC
R-PU-SD-OK	CATGGAATACCTCCTTTGTGCGACGTAAGCCACGTCAATCG
F-PL-O1-B	GCTACAGTTTAAACTGTAACAGGAGACGGTATGAGAAATCAATATAATACAC
R-PL-O1	CTCCTGTTACAGTTTAAACtgtAGcaagattgaatgttacagtttaaacTG
F-PL-SD-T	CAGTTATTATTGTAACAgTagacggtatgAGAAATCAATATAATACAC
R-PL-SD-T	GATTTCTCATACCGTCTACTGTTACAATAATAACtgttacaag
F-R6K-weapon	CAAGAGCCATCAATTCCCATGTCAGCCGTTAAGTG
R-R6K-weapon	GTAATACTGCGGCGTAGAGGATCTGAAGATCAGCAGTTC
F-weapon-R6K	GATCCTCTACGCCGCAGTATTACAAAAGGATGTCGCAAACGCTG
R-weapon-R6K	GGGAATTGATGGCTCTTGTATCTATCAGTGAAGCATC

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