Chromosomal Resistance to Metronidazole in *Clostridioides difficile* can be Mediated by Epistasis Between Iron Homeostasis and Oxidoreductases

Aditi Deshpande[1#], Xiaoqian Wu[1#], Wenwen Huo, Kelli Palmer[2], Julian G. Hurdle[1*]

[1]Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, Texas 77030, USA.

[2]Department of Biological Sciences, University of Texas at Dallas, Richardson, TX 75080, USA.

*Correspondence and requests for materials should be addressed to J.G.H (jhurdle@tamu.edu).

[#]These authors equally contributed. Author order was determined both alphabetically and in order of increasing seniority.

Table of contents

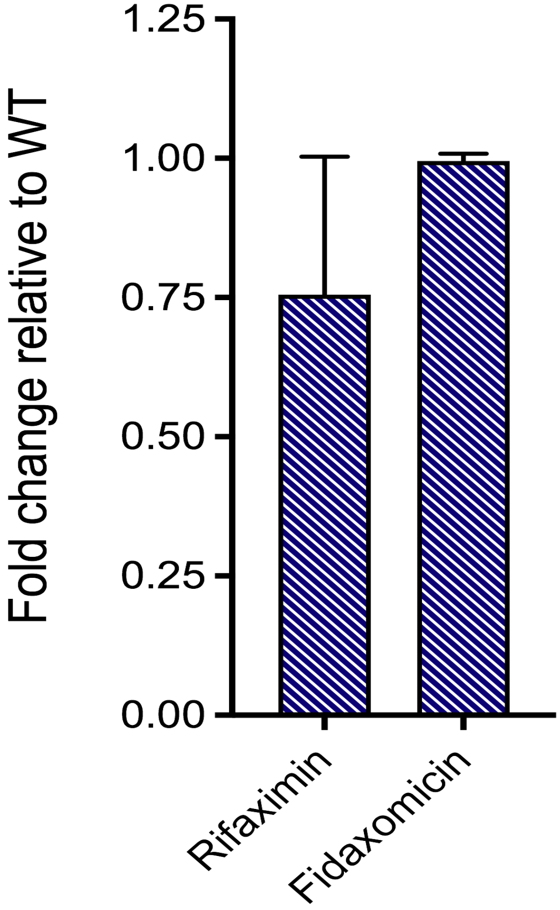


Figure S1. Complementation of the mutator reverses mutability. The mutator 700057Δ*mutSL* was complemented with WT *mutSL* on its own promoter in the vector pMTL-84151. Mutation frequencies of the complemented strain relative to the WT (~0.75 fold for rifaximin and almost equal for fidaxomicin) showed reversal of mutability.

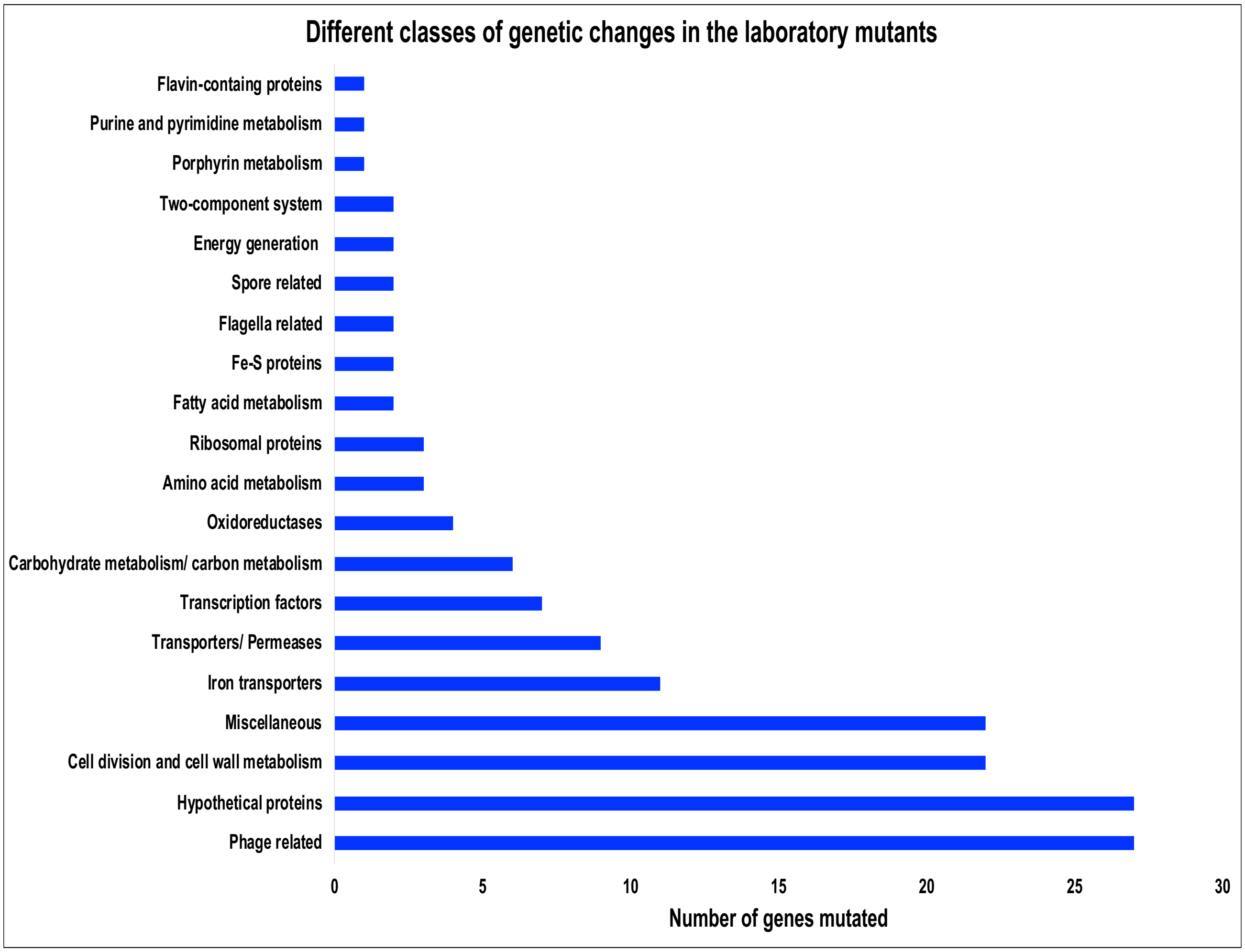


Figure S2. Functional categories of mutated genes found in the endpoint mutants (strains JWD-1 to JWD-4).

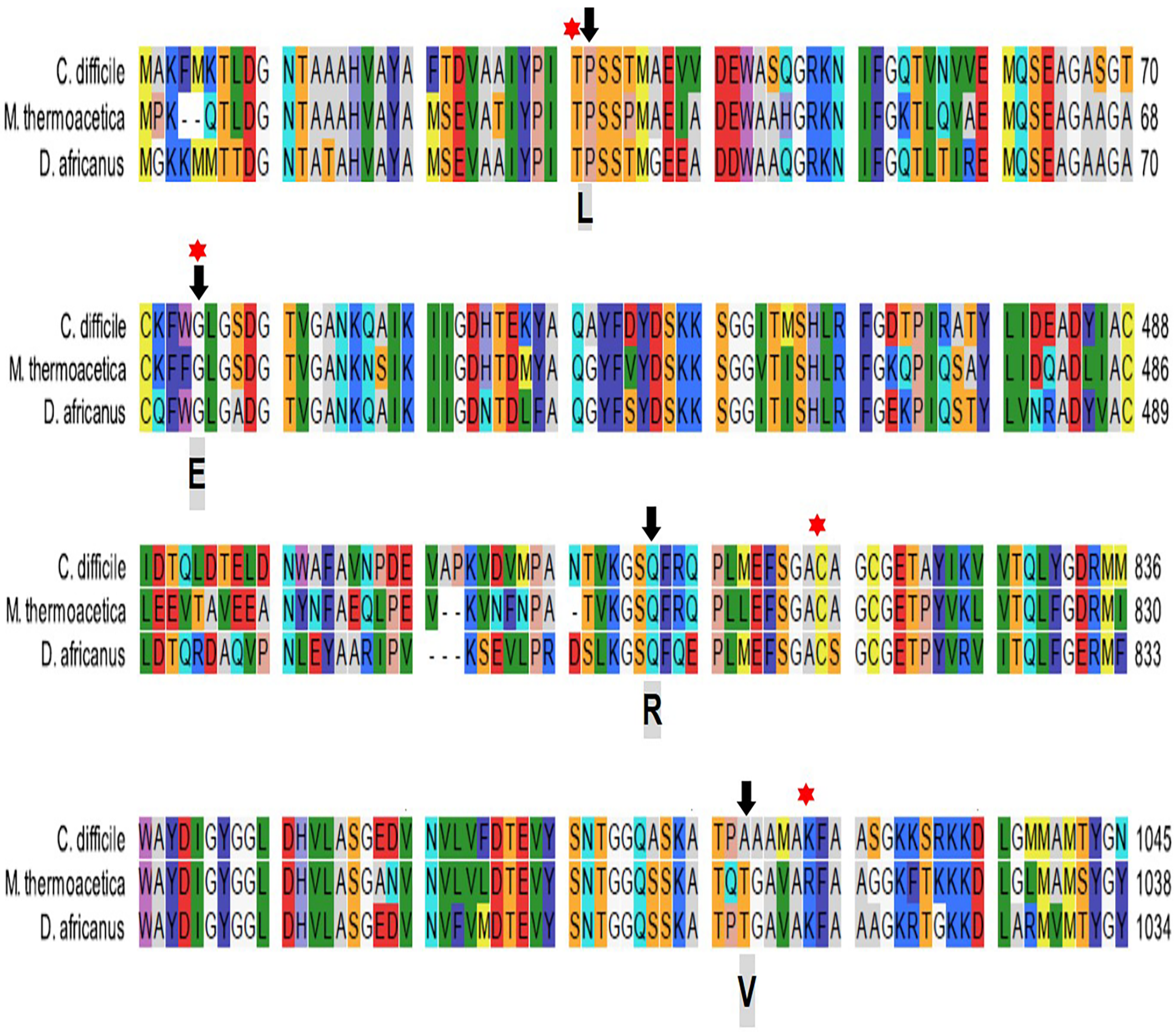


Figure S3. Alignments of PFOR of *C. difficile* (CD630_26820), *Moorella thermoacetica* (Q2RMD6) and *Desulfovibrio africanus* (Desaf_2186); substitutions are marked by black arrows and key conserved sites by red asterisks. In lab mutants**,** Pro_{32}Leu and Gln_{803}Arg lie close to critical Thr_{31} and Cys_{815} sites in PFOR catalytic domains. The Gly_{423}Glu in CDI strain CD26A54_R occurs in a conserve domain for coenzyme A binding, while Ala_{1018}Val in CDI strains 491858 or 490054 are in domain VI, near arginine-1016 in *M. thermoacetica* thought to stabilize coenzyme A by charge-charge interactions (structural analysis will be required to identify if *C. difficile* lysine-1023 has the same role in binding coenzyme A).

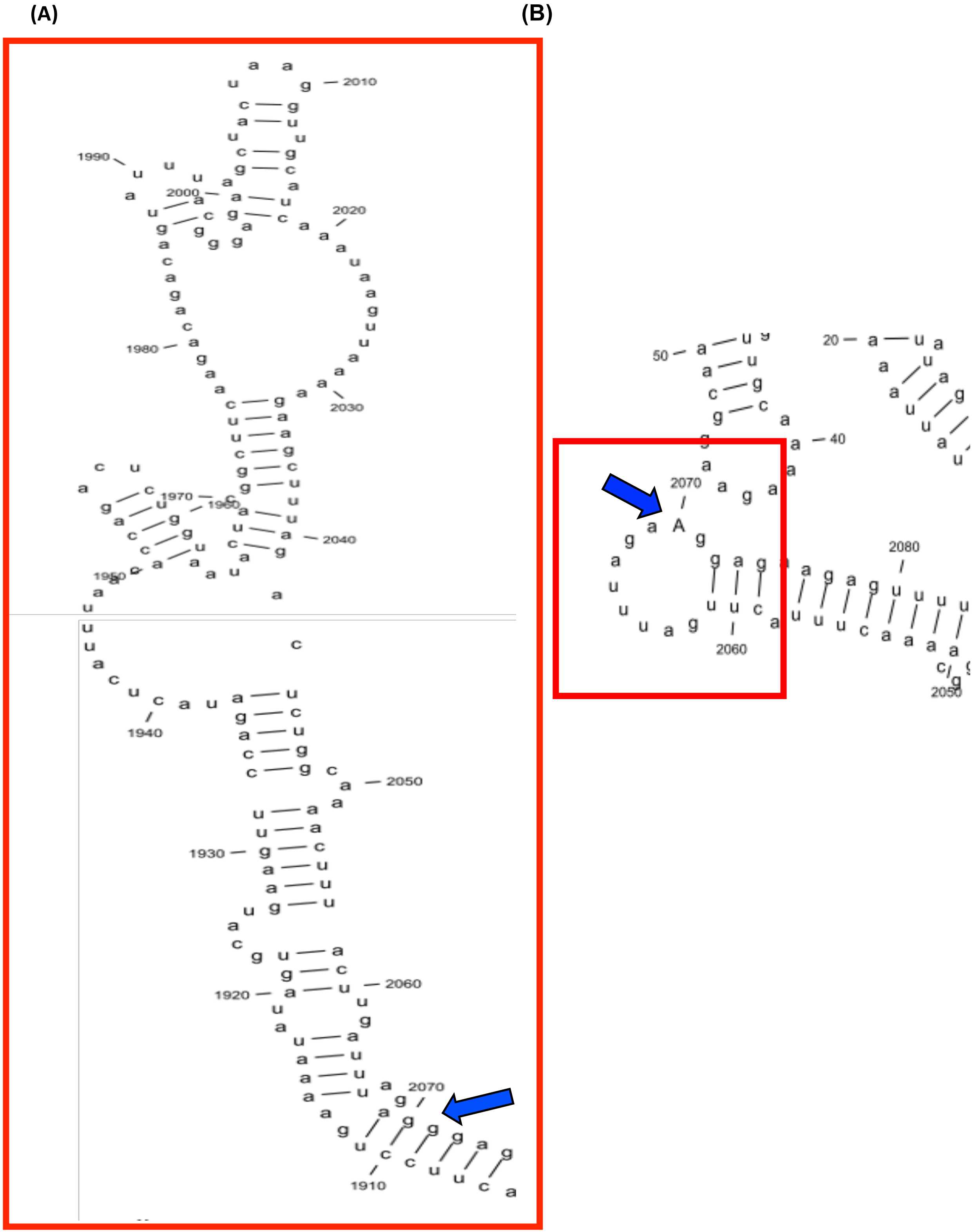


Figure S4. Analysis of xanthine dehydrogenase. **(A, B)** RNA secondary structure analysis of WT and mutant *xdh* (*CD630_31770*) was done using the mfold algorithm in CLC Genomics Workbench 12.0. We analyzed the entire mRNA of 2562 nucleotides from WT and mutant, which differed by the SNP (GA$\mathbf{G}_{2070}$GA**A**). Blue arrows indicate position 2070. (**A**) In the secondary RNA structure of WT and *xdh* the mutation site is part of a large loop region, with base pairing between 1910 and 2070, which a free energy change of ΔG= -25.9 kcal/mol. (**B**) The mutation impacted this region, producing a new loop region (2062-2071 bases) that was significantly more unstable (ΔG= 3.8 kcal/mol) and there was no base pairing with position 1910. In general, the more positive the free energy the more unstable the mRNA, which may affect transcription and translation. Although the mfold algorithm is an established method to analyze thermodynamic stability of RNA, its results might differ from actual biological stability in cells. Noteworthy, *CD630_31770* is annotated as *xdhA5* or *xdh* in CD630 and *xdhA3* in *CDR20291_3033* in R20291; herein to avoid confusion, it is referred to as *xdh*). CD630_31770 is predicted to be a molybdenum and iron-sulfur binding protein that is functionally related to oxidoreductase enzymes EC: 1.17.14 (xanthine dehydrogenase) and EC: 1.2.99.7 (aldehyde dehydrogenase). BLAST of CD630 genome shows there are 4 homologs of *CD630_31770* as follows: *xdhA1/CD630_20730*, with 27.20% identities; *xdhA2/CD630_20790*, with 27.62% identities; *xdhA3 /CD630_20870*, with 31.30% identities; *xdhA4/CD630_20990*, with 27.56% identities.

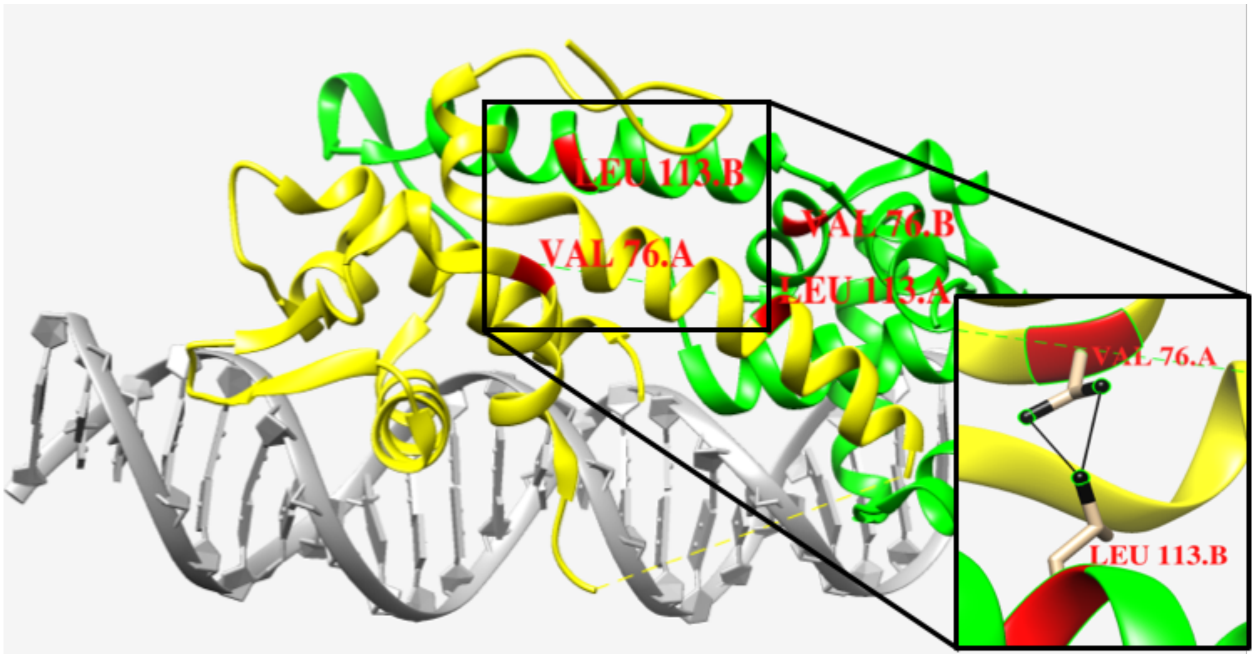


Figure S5. Structure of *E. coli* dimeric IscR bound to DNA (pdb ID: 4CHU) was analyzed using UCSF Chimera. Val-76 on chain A interacts with Leu-113 on chain B. Substitution of $Val_{76}Ala$ might lead to lesser hydrophobic Ala to reduce the DNA dimer-IscR complex stabilization.

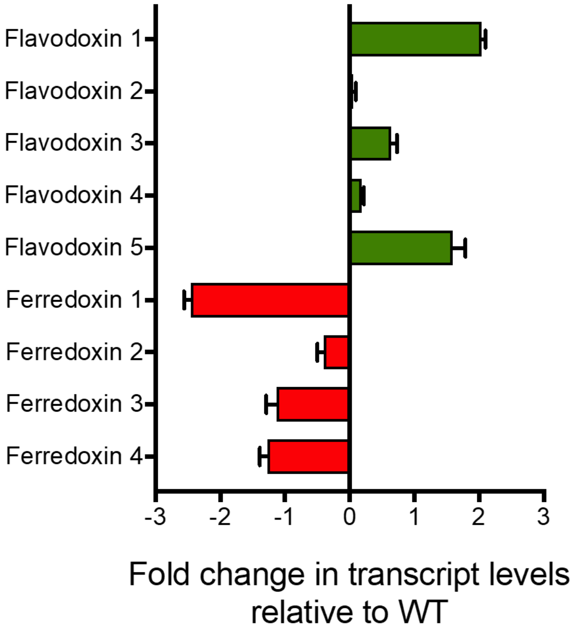
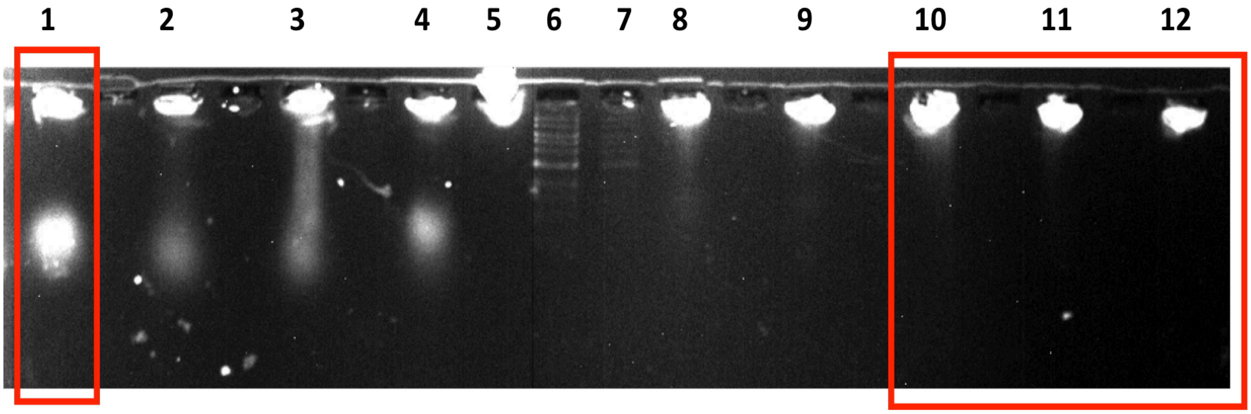
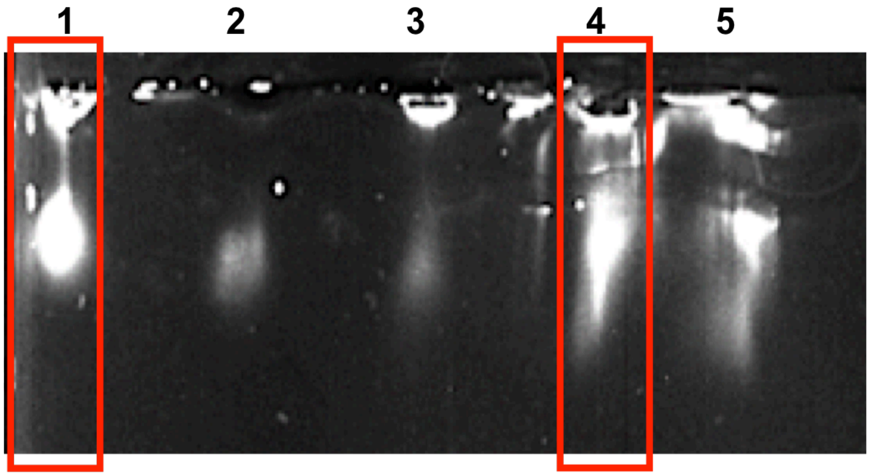


Figure S6. Analysis of flavodoxin (*fldx*) and ferredoxin (*fdx*) transcription as an indicator of cellular iron stress. In the absence of drug, compared to the parent WT, in the *feoB1* mutant all the flavodoxin homologs were upregulated, with flavodoxin 1 (CD630_19990) being the most expressed compared to flavodoxin 2 [CD630_28250], flavodoxin 3 [CD630_16790], flavodoxin 4 [CD630_31210] and flavodoxin 5 [CD630_22070]. All ferredoxin homologs were downregulated, with ferredoxin 1 (CD630_06271) being the most affected compared to others (ferredoxin 2 [CD630_36051], ferredoxin 3 [CD630_01721] and ferredoxin 4 [CD630_01150]).



Lane 1: ATCC Δ*feoB1*-empty vector – MTZ treated
Lane 2: ATCC Δ*feoB1* –empty vector- MTZ treated
Lane 3: ATCC- Hydrogen peroxide treated
Lane 4: ATCC- MTZ treated
Lane 5: ATCC Δ*feoB1* –*nifJi*- untreated
Lane 6: Ladder
Lane 7: Ladder
Lane 8: ATCC Δ*feoB1-iscRi* – untreated
Lane 9: ATCC Δ*feoB1* –*xdhAi*- untreated
Lane 10: ATCC- Δ*feoB1 nifJi*- MTZ treated
Lane 11: ATCC- Δ*feoB1-iscRi* – MTZ treated
Lane 12: ATCC Δ*feoB1* –*xdhAi*- MTZ treated



Lane 1: ATCC– MTZ treated
Lane 2: ATCC– MTZ treated
Lane 3: ATCC- MTZ treated
Lane 4: ATCC- Hydrogen peroxide treated
Lane 5: ATCC- Hydrogen peroxide treated

Figure S7. Original images of gels demonstrating DNA fragmentation caused by metronidazole or hydrogen peroxide. Lanes highlighted in red are those shown in the main manuscript. Shown are representative biological replicates; all gels above were run at the same time.

Table S1. Comparison of mutation frequencies for various strains; significance was established by unpaired t-tests versus WT ATCC700057 or Δ*mutSL* double mutant.

	Mutation frequencies against various strains (Average ± Std x 10^{-8})				
	WT	Δ*mutS2*	Δ*mutS*	Δ*muL*	Δ*mutSL*
			Rifaximin		
	2.64 ± 1.23	2.26 ± 0.81	71.1 ± 14.2	115 ± 2.37	208 ± 23.9
p value wrt WT	-	0.68	0.013	<0.0001	0.005
p value wrt Δ*mutSL*	-	0.005	0.003	0.021	-
			Fidaxomicin		
	3.37E ± 0.58	2.30 ± 0.3	69.8 ± 2.52	55.7 ± 6.73	313 ± 45.6
p value wrt WT	-	0.065	0.0003	0.005	0.007
p value wrt Δ*mutSL*	-	0.007	0.011	0.009	-

Table S2. Intragenic variations in metronidazole-resistant JWD-1, -2, -3 and -4.

- Yes or No in the last column indicates presence or absence of variation across the respective strains, as determined from genome analysis.
- Yellow highlight indicate the variations were found in Sanger sequences in Sanger sequences.
- Green highlight indicate the variation(s) were not found in Sanger sequences.

Gene ontology	**Overlapping annotations**	**Type**	**Nucleotide change**	**Amino acid change**	**Metronidazole-resistant mutants**			
					JWD-1	**JWD-2**	**JWD-3**	**JWD-4**
Iron transporters	Ferric iron ABC transporter	SNV	170G>A	Gly57Asp	No	Yes	No	No
	Ferrous iron transport protein B	Deletion	117delA	Glu40fs	No	No	No	**Yes**
	Ferric iron ABC transporter	SNV	123A>T	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	133C>T	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	103C>T	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	153G>A	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	55T>C	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	135A>G	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	87A>G	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	99T>C	-	No	Yes	Yes	No
	Ferric iron ABC transporter	SNV	126G>A	-	No	Yes	Yes	No
	Ferrous iron transport protein B	Insertion	117_118 InsA	Glu38fs	**Yes**	**Yes**	**Yes**	No
Oxidoreductases	Pyruvate-flavodoxin oxidoreductase	SNV	2408A>G	Gln803Arg	**Yes**	**Yes**	**Yes**	No
	Pyruvate-flavodoxin oxidoreductase	SNV	95C>T	Pro32Leu	No	No	No	**Yes**

	Xanthine dehydrogenase_ molybdenum binding subunit	SNV	2070G>A	-	**Yes**	**Yes**	**Yes**	No
	Benzoyl-CoA reductase subunit BadE	Deletion	8delA	Glu6fs	No	No	No	Yes
Flavin-containing proteins	Electron transfer flavoprotein_ alpha subunit	SNV	737G>A	Gly246Glu	No	No	No	Yes
Fe-S proteins	Iron-sulfur cluster regulator IscR	Deletion	153delA	Lys51fs	No	**Yes**	No	**Yes**
	Iron-sulfur cluster regulator IscR	SNV	227T>C	Val76Ala	No	No	**Yes**	No
Transcription factors	Beta-glucoside bgl operon antiterminator_BglG family	Deletion	97delA	Arg33fs	No	Yes	Yes	No
	Transcriptional antiterminator of lichenan operon_BglG family	SNV	177T>C	-	No	No	No	Yes
	Tn916_transcriptional regulator_putative	SNV	626G>A	Arg209His	Yes	No	No	No
	Xre family DNA-binding domain and TPR-repeat-containing protein	Insertion	923_924 insA	Asn308fs	No	Yes	Yes	No
	Transcriptional regulators of sugar metabolism	Deletion	118delA	Ile40fs	No	Yes	Yes	No
	NtrC family Transcriptional regulator_ATPase domain	Deletion	237delA	Thr79fs	No	No	Yes	No
	Transcriptional regulator_FUR family	Deletion	90delA	Asp33fs	No	No	No	**Yes**
Amino acid metabolism	3-dehydroquinate synthase	Deletion	97delA	Ile33fs	No	No	No	Yes
	Putative aminopeptidase	SNV	754G>A	Asp252Asn	No	No	No	Yes
	Histidinol-phosphate aminotransferase	SNV	390T>C	-	No	No	Yes	No

Carbohydrate/ Carbon metabolism	Acetate kinase	SNV	67G>A	Glu23Lys	No	No	No	Yes
	Alcohol dehydrogenase	SNV	606G>A	-	No	No	No	Yes
	Endoglucanase	SNV	939G>A	-	No	No	No	Yes
	Endoglucanase	Deletion	53delT	Leu18fs	No	No	No	Yes
	PTS system_fructose-specific IIB component	SNV	235G>A	Glu79Lys	No	No	No	Yes
	Similar to carbon monoxide dehydrogenase CooS subunit	SNV	740A>G	Tyr247Cys	Yes	Yes	Yes	No
Fatty acid metabolism	Enoyl-[acyl-carrier-protein] reductase [FMN]	SNV	341C>A	Pro114His	Yes	Yes	Yes	No
	Cardiolipin synthetase	Deletion	1198delA	Ile401fs	No	No	No	Yes
Porphyrin metabolism	Cobalt-precorrin-4 C11-methyltransferase	SNV	563T>C	Ile188Thr	No	No	No	Yes
Purine and pyrimidine metabolism	2',3'-cyclic-nucleotide 2'-phosphodiesterase	SNV	509T>C	Val170Ala	No	No	No	Yes
Transporters/ Permeases	ABC transporter permease protein	SNV	576A>G	-	No	No	Yes	Yes
	ABC transporter permease protein	SNV	540T>C	-	No	No	No	Yes
	ABC transporter permease protein	SNV	412T>C	Phe138Leu	No	No	No	Yes
	Amino acid ABC transporter_ATP-binding protein	Deletion	341delA	Glu114fs	No	Yes	Yes	No
	Xanthine permease	SNV	809G>A	Gly270Asp	No	No	No	**Yes**
	EcsC protein	SNV	60G>A	Trp20*	No	Yes	Yes	No
	ABC transporter permease protein	Deletion	11delA	Asn4fs	Yes	Yes	Yes	No
	ABC transporter permease protein	Deletion	327delA	Pro109fs	Yes	Yes	No	No
	Ethanolamine permease	SNV	987T>C	-	No	Yes	No	No

Flagella-related proteins	Flagellar hook-associated protein FlgK	SNV	1183G>A	Val395Ile	No	No	No	Yes
	Flagellar hook-length control protein FliK	SNV	2712G>A	-	No	No	No	Yes
Sporulation-related proteins	Putative small acid-soluble spore protein	Deletion	167delA	Asn56fs	No	No	No	Yes
	RNA polymerase sporulation specific sigma factor SigK	SNV	38C>T	Pro13Leu	No	Yes	No	No
Cell division and cell wall-related proteins	Cell division protein FtsH	SNV	840T>C	-	No	No	No	Yes
	N-acetylmuramoyl-L-alanine amidase	SNV	282C>T	-	No	No	No	Yes
	N-acetylmuramoyl-L-alanine amidase	SNV	132C>T	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	39_40 delGG insAC	Gly13_Ala14 del insGlyPro	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	13G>A	Val5Ile	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	152_153 delAA insCC	Lys51Thr	No	Yes	No	No
	N-acetylmuramoyl-L-alanine amidase	SNV	808T>C	-	No	Yes	Yes	No
	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	SNV	882A>G	-	No	Yes	No	No
	N-acetylmuramoyl-L-alanine amidase	SNV	33G>A	-	No	Yes	Yes	No
	Cell division protein FtsK	SNV	2058A>G	-	Yes	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	72A>G	-	No	Yes	Yes	No
	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase	SNV	13delA	Lys5fs	No	Yes	No	No
	N-acetylmuramoyl-L-alanine amidase	SNV	816T>C	-	No	Yes	No	No

	N-acetylmuramoyl-L-alanine amidase	SNV	60G>T	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	122_123 delCT insAA	Thr41Lys	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	150C>T	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	162T>C	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	168C>T	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	Insertion	87A>G	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	SNV	171A>G	-	No	Yes	Yes	No
	N-acetylmuramoyl-L-alanine amidase	Deletion	129T>C	-	No	Yes	No	No
	putative amidohydrolase	SNV	55_56 insA	Glu19fs	No	Yes	No	No
Ribosomal proteins	rRNA: rRNA	SNV		-	Yes	No	No	Yes
	DNA-directed RNA polymerase beta subunit	SNV	2104G>A	Ala702Thr	Yes	No	No	No
	COG1399 protein_clustered with ribosomal protein L32p	Deletion	257delT	Leu86fs	Yes	No	No	No
Two-component systems	Osmosensitive K+ channel histidine kinase KdpD	Insertion	37_38insA	Ile13fs	No	No	No	Yes
	Two-component sensor histidine kinase	SNV	652T>A	Leu218Ile	No	No	Yes	No
Phage related proteins	Phage antirepressor protein	SNV	401T>C	Val134Ala	No	No	No	Yes
	Phage tail fiber protein	SNV	228T>C	-	No	No	No	Yes
	Phage-like element PBSX protein xkdK	SNV	447T>A	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	441C>T	-	No	Yes	Yes	No

	Phage-like element PBSX protein xkdK	SNV	438T>A	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	420C>T	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	384C>T	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	375T>A	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	348_349 delGA insAG	Leu116_Ile 117del ins Leu Val	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	345C>T	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	300G>A	-	Yes	Yes	Yes	No
	Phage-related protein	SNV	612G>A	-	No	Yes	No	No
	Phage tail length tape-measure protein	SNV	1299G>T	-	Yes	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	358_360 delGTA insACT	Val120Thr	No	Yes	No	No
	Phage-like element PBSX protein xkdK	SNV	288T>C	-	Yes	Yes	Yes	No
	Phage replication initiation protein	SNV	708G>A	-	No	No	No	No
	Phage-like element PBSX protein xkdK	SNV	330A>G	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	312C>T	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	315_316 del GA ins AG	Lys105_Ile 106 del ins Lys Val	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	326T>C	Ile109Thr	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	570C>T	-	No	Yes	Yes	No

	Phage-like element PBSX protein xkdK	SNV	334G>T	Val112Leu	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	430A>G	Ile144Val	No	Yes	Yes	No
	Phage-related protein	SNV	609_610 del TT ins CC	-	No	Yes	No	No
	Phage-like element PBSX protein xkdK	Replacement	359_360 del TA ins ACT	Val120fs	No	No	Yes	No
	Phage-like element PBSX protein xkdK	SNV	381T>C	-	No	Yes	Yes	No
	Phage-like element PBSX protein xkdK	SNV	444T>C	-	No	Yes	Yes	No
Hypothetical Proteins	FIG00514869:_hypothetical protein	SNV	599C>T	Ala200Val	No	No	No	Yes
	FIG00515698:_hypothetical protein	SNV	2679G>A	-	No	No	No	Yes
	FIG00517149:_hypothetical protein	Deletion	21delA	Lys7fs	No	No	No	Yes
	FIG00518329:_hypothetical protein	Deletion	1461delA	Lys487fs	No	No	Yes	No
	FIG00518900:_hypothetical protein	SNV	ad4A>G	Thr62Ala	No	No	No	Yes
	Hypothetical protein	SNV	60G>A	Trp20*	No	Yes	Yes	No
	Hypothetical protein	SNV	60T>C	-	No	0	0	Yes
	Hypothetical protein	SNV	244T>C	82Gln*	No	0	0	Yes
	FIG00514672:_hypothetical protein	SNV	204T>C	-	No	Yes	Yes	No
	FIG00521667:_hypothetical protein	SNV	1006T>C	Cys336Arg	No	Yes	0	No
	FIG00513672:_hypothetical protein	SNV	920C>T	Pro307Leu	No	0	0	Yes
	FIG00518735:_hypothetical protein	SNV	391G>A	Asp131Asn	No	0	0	Yes

	FIG00516442:_hypothetical protein	SNV	54G>A	-	No	0	0	Yes
	FIG00516222:_hypothetical protein	SNV	393T>C	-	Yes	Yes	Yes	No
	FIG00515235:_hypothetical protein	Deletion	519delA	Ile173fs	Yes	Yes	Yes	No
	FIG00512719:_hypothetical protein	SNV	339T>C	-	Yes	Yes	Yes	No
	FIG00519548:_hypothetical protein	SNV	559T>C	Ser187Pro	No	Yes	Yes	No
	FIG00516776:_hypothetical protein	SNV	126T>C	-	No	Yes	No	No
	FIG00514341:_hypothetical protein	Deletion	1610delA	Asn537fs	No	Yes	Yes	No
	FIG00514462:_hypothetical protein	Deletion	1184delA	Asn395fs	No	Yes	Yes	No
	FIG099352:_hypothetical protein	Deletion	725delA	Lys242fs	Yes	Yes	Yes	No
Energy-generation proteins	ATP synthase alpha chain	SNV	1228C>T	Leu410Phe	Yes	Yes	Yes	No
	ATP synthase alpha chain	SNV	892C>T	Leu298Phe	No	No	No	Yes
Miscellaneous	FIG060329:_MOSC domain protein	SNV	227G>A	Gly76Glu	No	No	No	Yes
	FIG146085:_3'-to-5' oligoribonuclease A, Bacillus type	SNV	131G>T	Arg44Met	No	No	No	Yes
	Putative membrane protein YeiH	SNV	821C>T	Ala274Val	Yes	Yes	Yes	No
	putative phage membrane protein	SNV	237A>G	-	No	Yes	Yes	No
	RNA methyltransferase_TrmA family	SNV	449A>G	Lys150Arg	No	No	No	Yes

	tRNA uridine 5-carboxymethyl-aminomethyl modification enzyme GidA	SNV	380T>C	Val127Ala	No	No	No	Yes
	Membrane protein_putative	SNV	685C>T	Pro229Ser	No	No	No	Yes
	Putative phage membrane protein	SNV	240A>G	-	Yes	Yes	Yes	No
	Putative phage membrane protein	SNV	252T>C	-	No	Yes	Yes	No
	Probable oligoendopeptidase F	SNV	332A>G	Gln111Arg	No	Yes	Yes	No
	Late competence protein ComEC_DNA transport	SNV	785T>C	Met262Thr	No	Yes	Yes	No
	Antirestriction protein	SNV	344A>G	Asp115Gly	Yes	Yes	Yes	No
	Serine-protein kinase RsbW	Deletion	193delT	Phe65fs	Yes	Yes	Yes	No
	Similar to terminase small subunit_yqaS homolog	Deletion	192delA	Gly65fs	No	No	No	No
	Resolvase-like:Recombinase	Insertion	726_727 insA	Gln243fs	No	Yes	Yes	No
	Pantoate--beta-alanine ligase	Insertion	119_120 insA	Ala43fs	No	No	No	Yes
	Mobile element protein	SNV	132G>T	-	No	No	No	Yes
	D-aminoacylase	SNV	916C>T	-	No	No	No	Yes
	Metallo-dependent hydrolases_subgroup B	SNV	337A>T	Lys113*	No	No	No	Yes
	Nitrogen regulation protein NtrX	SNV	666G>A	-	No	No	No	Yes
	Ornithine cyclodeaminase	SNV	438G>A	Met146Ile	No	No	Yes	No
	Putative response regulator; homolog of RumR and ScnR	SNV	204T>C	-	No	No	No	Yes

Table S3. Genetic changes in the clinical isolates

- Yes or No in the last column indicates presence or absence of variation across the respective strains, as determined from genome analysis.
- Yellow highlight indicate the variations were found in Sanger sequences.

Genes	Annotations	Clinical strains			
		491858		495004	
		Nucleotide change	Amino acid change	Nucleotide change	Amino acid change
Pyruvate-ferredoxin oxidoreductase (PFOR)	CDR20291_2570	3053C>T	Ala1018Val	3053C>T	Ala1018Val
Putative 5-nitroimidazole reductase (Nim)	CDR20291_1308	389A>C	Tyr130Ser	389A>C	Tyr130Ser
Putative 5-nitroimidazole reductase (Nim)	CDR20291_1308	-	-	463A>T	Ile155Leu
Putative ruberythrin (Rbr)	CDR20291_1323	412C>A	Gln138Lys	412C>A	Gln138Lys
Quinolinate synthase (NadA)	CDR20291_2259	205C>A	Leu69Ile	205C>A	Leu69Ile
Electron transfer flavoprotein beta-subunit (EtfB)	CDR20291_0735	31T>C	Ser11Pro	31T>C	Ser11Pro
Response regulator VanR	CDR20291_1522	343A>G	Thr115Ala	343A>G	Thr115Ala
UDP-N-acetylmuramoyl-alanine-D-glutamate ligase (MurD)	CDR20291_2541	902C>T	Thr301Ile	902C>T	Thr301Ile
RNA polymerase B (RpoB)	CDR20291_0060	1514G>A	Arg505Lys	1514G>A	Arg505Lys
Recombination protein (RecA)	CDR20291_1169	-	-	28G>A	Ala10Thr

Table S4. Bacterial strains and plasmids.

Strains	Description	Reference/ Source
E. coli		
NEB 5- alpha	*fhuA2 ∆(argF-lacZ) U169 phoA glnV44 Φ80 ∆(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	*New England Biolabs*
CA434	*E. coli* HB101 with plasmid R702	22
SD46		Dr. Robert Britton, Baylor College of Medicine, Houston
C. difficile		
ATCC 700057	Wild type, *tcdA*$^{-}$ *tcdB*$^{-}$, Ribotype 038	ATCC
ATCC 700057 Δ*mutS*	Δ*mutS* deletion	This study
ATCC 700057 Δ*mutL*	Δ*mutL* deletion	This study
ATCC 700057 Δ*mutS2*	Δ*mutS2* deletion	This study
ATCC 700057Δ*mutSL*	Δ*mutSL* deletion	This study
ATCC 700057Δ*mutSL*:: *pmutSL*	Δ*mutSL* complemented with pMTL84151- *mutSL*	This study
ATCC 700057Δ*feoB1*	Δ*feoB1* deletion	This study
ATCC 700057Δ*feoB1*:: p*feoB1*	Δ*feoB1* complemented with pMTL84151-*feoB1*	This study
491858 and 490054	Clinical isolate	(1, 2)
CD26A54_R	Clinical isolate	(3)
491858::Empty vector	491858 carrying plasmid pMTL84151.	This study
491858::p*nifJ*	491858 carrying plasmid pMTL84151 with *nifJ* driven by its own promoter.	This study
CD26A54_R::Empty vector	CD26A54_R carrying plasmid pMTL84151.	This study
CD26A54_R::p*nifJ*	CD26A54_R carrying plasmid pMTL84151 with *nifJ* driven by its promoter	This study
Plasmids	**Description**	**Reference/ Source**
pMTL-SC7215	*E. coli- C. difficile* shuttle vector (pBP1 *catP* ColE1 *traJ codA*)	(4)
pMTL84151	*E. coli- C. difficile* shuttle vector	(5)

pMTL84151-*mutSL*	pMTL84151 with *mutSL* genes and promoter	This study
pMTL84151-*feoB1*	pMTL84151 with *feoB1* gene and promoter	This study
pMTL84151-*nifJ*	pMTL84151 with *nifJ* gene and promoter	This study
pXWpxyl-dcas9	Xylose-inducible vector for gene knockdown	(6)
pXWpxyl- *feoB1*- AS	Xylose-inducible knockdown for *feoB1*	This study
pXWpxyl- *nifJ* - AS	Xylose-inducible knockdown for *nifJ*	This study
pXWpxyl- *iscR* - AS	Xylose-inducible knockdown for *iscR*	This study
pXWpxyl- *xdh* - AS	Xylose-inducible knockdown for *xdh*	This study

Table S5. Primers used in this study.

Primer Name	**Description**	**Sequence**
For Sanger sequencing		
feoB1-F	To confirm the mutations Glu38*fs* and Lys40*fs*	TGTAAAACGACGGCCAGTGGTGACCCAATTGAGGTAACAGTC
feoB1-R		CAGGAAACAGCTATGACGCCTTTTCAGCAGCTTCCATAATTC
nifJ-F	To confirm the mutation Pro32Leu	TGTAAAACGACGGCCAGTGAAGATAGGTATTTGCGCGAC
nifJ-R		CAGGAAACAGCTATGACGCAGCCATAACGTCTTGGTG
nifJ-F	To confirm the mutation Gln803Arg	TGTAAAACGACGGCCAGTGGTACTGCAGCTTATGAAAAACGTGG
nifJ-R		CAGGAAACAGCTATGACCTTAGCACTTGCTACTTTAGAAGCTTCTCC
iscR-F	To confirm the mutation Lys51*fs* and Val76Ala	TGTAAAACGACGGCCAGTGTTTGTTTTATGATAATGAAGAGGC
iscR-R		CAGGAAACAGCTATGACCTAATTTATTTTTGTTATAGTCATTTATCATATC
xdh-F	To confirm the mutation GAG2070GAA	TGTAAAACGACGGCCAGTGAGTTGGTATACCTGATGTTGG
xdh-R		TCAGGAAACAGCTATGACCTCGGTTAGAGCATATCCAAGAC
Xanthine permease-F	To confirm the mutation Gly270Asp	TGTAAAACGACGGCCAGTCTATTAGAGAGGCAACTTGGTTTG
Xanthine permease-R		CAGGAAACAGCTATGACGCACAAATCTACTTATTACTTTGTTCATAG
For Gene Deletions		
up-*mutS*-F	For fusion PCR to delete *mutS*	ATCTGGATCCGAAGCTATAATGCTATCAAACAG
up-*mutS*-R+	For fusion PCR to delete *mutS*	GCATCTAATGGAGATACTGTTTCATCATAGGAG
down-*mutS*-F+	For fusion PCR to delete *mutS*	GATGAAACAGTATCTCCATTAGATGCTATAAACTCTC
down-*mutS*-R	For fusion PCR to delete *mutS*	ATCTAAGCTTTGGTAGTTCCATTAGTAGAACC
up-*mutS2*-F	For fusion PCR to delete *mutS2*	ATCTGGATCCCCCATGACTCATGTTCCTCC
up-*mutS2*-R+	For fusion PCR to delete *mutS2*	CCACCTTCTCCCTAAAGAAGATGAGGCTTTCTTC
down-*mutS2*-F+	For fusion PCR to delete *mutS2*	CTCATCTTCTTTAGGGAGAAGGTGGAGCAGGAG

down-*mutS2*-R	For fusion PCR to delete *mutS2*	ATCTAAGCTTTTTCTTGTACTATCCAGTTGAC
down-*mutSL*-R+	For fusion PCR to delete *mutSL*	CAGCAGCAGCAGCAGCAGAGATACTGTTTCATCATAGGAG
up-*mutL*-F	For fusion PCR to delete *mutL*	ATCTGGATCCCAACAGTGTTGCAATAAATGGAG
up-*mutL*-R+	For fusion PCR to delete *mutL*	CAGCAGCAGCAGCAGCAGCTGCTGCTATCTTATTTATAGTC
down-*mutL*-F+	For fusion PCR to delete *mutL*	CTGCTGCTGCTGCTGCTGTAATGGTCGAAATATCTAAGACAG
down-*mutL*-R	For fusion PCR to delete *mutL*	ATCTAAGCTTAACTGAAAATGGATAGTCAGGC
Δ*mutS*-F	For PCR confirmation of *mutS* deletion	AAAACATCCATGCAAAAATGGC
Δ*mutS*-R	For PCR confirmation of *mutS* deletion	AAAAATTTCTGTCTTGCTGGTG
Δ*mutS2*-F	For PCR confirmation of *mutS2* deletion	TTCATTTCCAGAACCTCTAGG
Δ*mutS2*-R	For PCR confirmation of *mutS2* deletion	CTATAATTGCAGTTTTTAATCACAC
Δ*mutL*-F	For PCR confirmation of *mutL* deletion	GGAGCAGACAAGAGTTATGG
Δ*mutL*-R	For PCR confirmation of *mutL* deletion	TGCCTCCAGTTACTAAAACAC
feoB1-F	To confirm deletion of *feoB1*	AGGTGAAGAAGTAGTTATAAAGAAAATTTCGGG
feoB1-R	To confirm deletion of *feoB1*	CTAATAATGCTCTTCCCAATCCACCGC

Primer Name	**Description**	**Sequence**
For gene complementation		
mutS-F	For complementing Δ*mutSL* mutant	ATCTCTCGAGCCATTAGATGCTATAAACTCTC
mutL-R	For complementing Δ*mutSL* mutant	ATCTGAATTCAACTGAAAATGGATAGTCAGGC
nifJ-F	For complementing clinical strains	AAAGAGCTCTAAAAAACTTACAAAAATATGATG
nifJ-R	For complementing clinical strains	AAAGGATCCGGCAAGATGGAAAAC
For RT-qPCR experiments		
feoB1-qPCR-F	To analyze the expression of *feoB1*	GGT CTC ATA GGA AAT CCT AAC TGT GGT AAG ACA ACA ATG TTC AAT GGG
feoB1-qPCR-R	To analyze the expression of *feoB1*	CCTTTTTTTTCAACTGTTACTCCTGGCCAGTTACCAACATATTGTGATG
fhuB-qPCR	To analyze the expression of *fhuB*	GCTGTACTTGTATTTGGAATTGTATTATC

-F		CATAACTATTGG
fhuB-qPCR -R	To analyze the expression of *fhuB*	CAAGGGCAATAGCAGCTGCACTTGTTG
fdx-qPCR-F (CD630_06271)	To analyze the expression of *fdx*	GCAAATATTTTAGCAAATGAGTGCG
fdx-qPCR-R (CD630_06271)	To analyze the expression of *fdx*	GCACTAATGCCACATGGAACTGAGATAGC
fdx-qPCR-F (CD630_36051)	To analyze the expression of *fdx*	GAAAGCATTTGTTGATAAGGATGTATGTG
fdx-qPCR-R (CD630_36051)	To analyze the expression of *fdx*	CTGTTATAGCTGAAACTGGACATCCTTC
fdx-qPCR-F (CD630_01721)	To analyze the expression of *fdx*	CATATAAAATTACTGACGCTTGTATAAGCTGTG
fdx-qPCR-R (CD630_01721)	To analyze the expression of *fdx*	CTGGTTGAGGTGCATCTACAGG
fdx-qPCR-F (CD630_01150)	To analyze the expression of *fdx*	GCAAATGTTAATCAAGATACTTGTATAGGTTG
fdx-qPCR-R (CD630_01150)	To analyze the expression of *fdx*	CAACTGGACAGCTTTCTCTAGCTTC
fldx-qPCR-F (CD630_19990)	To analyze the expression of *fldx*	GGAGTGGAACAGGAAATACTGAAAAAATGGCAAATTTTGTGGCTG
fldx-qPCR-R (CD630_19990)	To analyze the expression of *fldx*	CTCCCATAGATGGGCAACCTAATGC
fldx-qpCR-F CD630_28250)	To analyze the expression of *fldx*	GATGAAATTCAAGAAGAAAAAGACATTAAAG
fldx-qpCR-R (CD630_28250)	To analyze the expression of *fldx*	GCACTTGTTTTGAATTTTTATCACAAATTC
fldx-qpCR-F CD630_16790)	To analyze the expression of *fldx*	GATAATGTAGAGTTATTAAACAAAAAACAAGTTGGC
fldx-qpCR-R (CD630_16790)	To analyze the expression of *fldx*	GATATTCTAAAACTGTCATAATTACCTTTTGAAAC
fldx-qpCR-F CD630_31210)	To analyze the expression of *fldx*	GATATGTGGCTCGTAAAATTGCAC
fldx-qpCR-R (CD630_31210)	To analyze the expression of *fldx*	CTATTGGCAATCTCCATGCATAAG
fldx-qpCR-F CD630_22070)	To analyze the expression of *fldx*	GAGGTCTTTATGCTTGTAATATAGCGGG
fldx-qpCR-R (CD630_22070)	To analyze the expression of *fldx*	CTTCTCTTAATTTCTTTATTACTTCATCATCATATGG
fur-qPCR-F	To analyze the expression of *fur*	ACGCCACAAAGAAGAGCAAT
fur-qPCR-R	To analyze the expression of *fur*	TCAGGACAGTCAACCCTAACT

nifJ-qPCR-F	To analyze the expression of *nifJ*	GGCTAAGTTTATGAAAACACTTGATGGA A ATACAGCTGCAGCTCATG
nifJ-qPCR-n-R	To analyze the expression of *nifJ*	CCCACTCATCAACTACTTCAGCCATAGT TGAAGATGGTGTG
iscR-qPCR-F	To analyze the expression of *iscR*	CGGGTCTAGTAA AAA GTG TGA GAG GTGCTCAAGGAGG
iscR-qPCR-R	To analyze the expression of *iscR*	CACATACATCTTCATCTAGTACACAGTC AGATAATGCAACTGGTCCTTC
xdh-qPCR-F	To analyze the expression of *xdh*	GTTGCAAAAGAAGGCAAAAAACTTTTAG
xdh-qPCR-R	To analyze the expression of *xdh*	GAACTAATTACCATACCTGGAATACAAA ATCC
recA-qpCR-F	To analyze the expression of *recA*	TGCAAAAGCTTTGGGTGTGG
recA-qpCR-R	To analyze the expression of *dnaK*	GCCTTTGGAACTAATGCTGCT
dnaK-qpCR-F	To analyze the expression of *dnaK*	ATCCCCTGCTAAAACTCCAGC
dnaK-qpCR-R	To analyze the expression of *dnaK*	ACCAGCAGTACAAGAAGCTGTT
hcp-qpCR-F	To analyze the expression of *hcp*	TGGAGGTTGTGATGGTGCAA
hcp-qpCR-R	To analyze the expression of *hcp*	AGCCACCTATATCCCCAAGGT
norV-qpCR-F	To analyze the expression of *norV*	TTGGTTGGAGTGGTGAAGGT
norV-qpCR-R	To analyze the expression of *norV*	GCAAATTCTGCACCAAATGCC
trx-qpCR-F (CD630_30330)	To analyze the expression of *trx*	ATTTCTTTGCGACTTGGTGTGG
trx-qpCR-R (CD630_30330)	To analyze the expression of *trx*	TCCACCTTCAAGAAGTTTGCT

Table S6. Gene knockdown, gene deletion and gene complementation constructs.

a) sgRNA targeting *feoB1*

GTTTAAACTAAGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTAT
TATTAGTTCAATTGTTGTCTTACCACAGTTGTTTTAGAGCTAGAAATAGCAAGTTAAA
ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTA
AGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTATTATTAGTTCA
GGAGATAAAGAATAAATACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTAAAC

b) sgRNA targeting *nifJ*

GTTTAAACTAAGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTAT
TATTAGTTCATGCTACATCTGTAAACGCATGTTTTAGAGCTAGAAATAGCAAGTTAA
AATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT
AAGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTATTATTAGTTC
ACTCCTCCTTGTAAAGAACCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT
AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTAAAC

c) sgRNA targeting *iscR*

GTTTAAACTAAGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTAT
TATTAGTTCAGCAATAAATTTTAATGATACGTTTTAGAGCTAGAAATAGCAAGTTAAA
ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTA
AGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTATTATTAGTTCA
ACACAGTCAGATAATGCAACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTAAAC

d) sgRNA targeting *xdh*

GTTTAAACTAAGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTAT
TATTAGTTCATTATATTCATATCTTCTCTAGTTTTAGAGCTAGAAATAGCAAGTTAAA
ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTA
AGTTAGTTTAATATACTAACAAATTGACAAAGATAATTAAATATTTTATTATTAGTTCA
TTTGAACTAATTACCATACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTAAAC

d) *feoB1* deletion fragment

GTTTAAACAGGTGAAGAAGTAGTTATAAAGAAAATTTCGGGAAGTGATAAGACGAG
GACTTTTTTAAATAGTATAGGTTTTGTAGTGGGTACAAATGTTAAAATAGTTTCTAAG
ATAGATGGTAACTTAATAATAGACGTAAAAGATACTAGAGTTGCATTAGATAAAAAA
ATGGCAAGTAGAATAATAATATAGGGAGGTAATGACATGAACAGACTGAAGGATAT
TAAGTGTGGAGAGACTGTAAAAGTCAAAAAGTTAGAAGGAGAAGGAGCTACACGA
AGACGTATAATGGATATGGGGATTACAAGAGGTGTTAATATATTTATAAGAAAAGTA
GCTCCTCTTGGTGACCCAATTGAGGTAACAGTCAGAGATTATGAACTATCAATTCG
TAAGTCAGATGCAGAAAAAATTATTGTGGAATAATAAAGATTAATTAGAGTAGGGTT
AATGTAATTATATAATGAGAATGATTTTCTGTATCACAAACAGTGATGTATTTAAATA
ATAATGGAGGGAATGGAAATGTCAATAAAAATAGGTCTGTTATATGAAAAATTAATT
CATATAAATAAGGAGTGATGAATATGGCTACATTTATTATAGCAGCTATTGTAGTAG
TATTAATGGCACTAGCTGTAATGTATATGGTTAAAAATTCAAAAAAGAATGGTAGCA
GTTGTGGATGTGGTTGTTCTGGATGCTCAAGTAGTAAGAGTTGTCATAGCGCTAAG
AGATAAATAGGATAAGAGCCTGATAAAAAGTTGAATTGCTTTATTATCAGGTTCTTA
TTTTTTGTATAAATAACAAATTTCATATTTGAAGTAGGTTAGACTATGCTGTAATTTTA

CTGTATAATTATATATAAATATATGATTAGGAGGGATTATTGCTAAATTAAGCAATAT
AAGTGAATATTATGTTTAAATTTGATGTTACAAACGAGCTTATGTGTATAGCAAGGG
GAAGTGGTAAATTTTTTGCAAAAAAAGGTGCTATGGTAGCCTTTAAAGGAAATTTTA
ATTTTGAAAAATTGCTGTTAGGTCCAAGTAATGGCGGTGGATTGGGAAGAGCATTA
TTAGGTTTAAAC

e) *feoB1* complementation fragment
CCCGGGTATTATTTTAATAGATTAAGATTTAAGGTATTGTAATTTATATATAAGATAA
CGATTGCTTGTAGATTTAAATTACTTTTAATGGAGGGAATGGAAATGTCAATAAAAA
TAGGTCTCATAGGAAATCCTAACTGTGGTAAGACAACAATGTTCAATGGGCTTACA
GGCTCATCACAATATGTTGGTAACTGGCCAGGAGTAACAGTTGAAAAAAAAGGTGG
AAAATTAAAAGGAAATAAAGATGTGGAAATAGTAGATTTACCAGGTATTTATTCTTTA
TCTCCATATACATTAGAAGAAGTAGTTACACGTAATTTTATGCTAGATGATAAGCCG
GATGCAGTAATAAATATTGTAGATGCATCAAATATAGAGAGAAATTTATATCTTACAA
CTCAAGTTTTAGAGCTAGGTATACCGACCGTTATAGCTCTTAATATGATGGATATTG
TAAATAAGAATGGTGATAAGATAAATATTAAAGAACTATCTGAAGTTATAGGTTGCC
CAGTGGTAGAAGTAACAGCAGTTAAAGGTCAAGGAATTATGGAAGCTGCTGAAAAG
GCAGTTGAACTTGCAAGTAGTAACAATAAATTAAATTTCAAATTACCCTTTGTTGAT
GAAAGTAAGGATGCAATAGAAAAAATTGAAAAAATAATAGAAGAAAAGACTCCATAT
ATAGATGTAGAAACACGCTGGTTAGCTATAAAGCTATTTGAGAGAGATGAAAATGTT
ATACAAAAACTTAATATTTCTAAAACAATATTGAATAGTATTGAAGAAATAACTAGAA
ATTGTGAAGATGAATTGGATGATGATAGTGAAAGTATAATAACAGCCAATCGTTATG
AATTTATTAGTTCAATAATTTCAAGTATAATAAAGAAGAATCGAAAAGGTAAGGAAA
CTGTATCAGATAAGATAGATAAGATTGTAACAAATCGTATACTAGCATTACCTATTTT
TGCACTTATAATGTGGGGAGTTTACTATATAGCAGTAAGTTCATTGGGTACTATAGC
AACTGATTGGACAAATGATGTACTGTTTGGAGAAATTATTCAAGGCAATGTAAGTAA
TTTTCTAGCTTCTTTAAATGTAGCAGAGTGGTTACAAGGACTTGTAGTTGATGGTCT
AATTGGTGGAGTTGGAGCAGTACTAGGATTTGTACCACAAATCATGCTTTTATTCTT
ATTATTATCAATATTAGAAGATTGTGGGTACATGTCACGTGTAGCTTTTATAATGGAT
AGAATTTTCCGTAAGTTTGGTCTTTCAGGAAAATCATTTATACCAATGCTTATAAGTT
CAGGTTGTGGAGTTCCAGGAGTTATGTCAACTCGTACTATAGAGAATGATAGAGAT
AGAAAAATGACTATTATGTTAACTACATTCATTCCATGTGGAGCTAAGATTCCAATA
ATAGCATTATTTGCAGGGGCATTATTTGGAGGTGCTTCATGGGTAGCTCCATCAAT
GTATTTCTTAGGTATTGCAATGATAATCATTTGTGGAATTATATTAAAGAAAACTAGT
TTATTTGCAGGAGAACCATCTCCATTTGTTATGGAATTACCTCAATATCATATTCCAA
GTGCAAAAGGTGTTTTAATACATATGTGGGATAGAGGAAAAGCATTTATAATCAAAG
CAGGAACAATTATATTTGTTGCTTGTGGAGTAATCTGGTTCTTACAATCATTTAATTG
GTCATTACAAATGGTAGATGCTGGAGATAGTATATTAGCTAGCTTAGGAAATATAGT
TGCACCAATATTTGCTCCTCTTGGATTTGGAAATTGGCAATCATCAGTAGCAACAGT
TACAGGTCTAGTAGCTAAGGAAAATGTAGTTGGAACATTTGGAGTTTTATTTGGTAT
ATCAGATGCAACAGAGCAAGACCCAACACTTCTTGCTTCAGTTGCAAGCATGTTTA
CAGTAGCTAGTGCGTTTGCATTTATGGCATTTAATATGCTTTGTGCACCTTGTTTTG
CAGCTATTGGAGCCATAAAAAGAGAAATGGGTTCATGGAAATGGACATGGATTACA
TTAGGATTCCAAACTTTAACAGCGTATATAATTGCTCTTCTTATAAATCAAGTTGGAA

GTTTAGTTTTAGGTACTGGAGGAAGTATTGCTGGGGCAATAATATCTATATTTATAG
CAGTAGCAGTAGTCTTTGTAGTTTTAACTTATTCAAATAAAAATATGAAAAAAGAAAA
AATGGGTAAATTAAGTTATATGAAAAATTAATCTAGA

Table S7. List of accession numbers for genomes of strains deposited in NCBI.

Strain name	BioSample Accession Number
JWD-1	SAMN13506423
JWD-2	SAMN13506424
JWD-3	SAMN13506425
JWD-4	SAMN13506426

References

1. Adler A, Miller-Roll T, Bradenstein R, Block C, Mendelson B, Parizade M, Paitan Y, Schwartz D, Peled N, Carmeli Y, Schwaber MJ. 2015. A national survey of the molecular epidemiology of *Clostridium difficile* in Israel: the dissemination of the ribotype 027 strain with reduced susceptibility to vancomycin and metronidazole. Diagn Microbiol Infect Dis 83:21-4.
2. Shen WJ, Deshpande A, Hevener KE, Endres BT, Garey KW, Palmer KL, Hurdle JG. 2020. Constitutive expression of the cryptic vanGCd operon promotes vancomycin resistance in *Clostridioides difficile* clinical isolates. J Antimicrob Chemother 75:859-867.
3. Lynch T, Chong P, Zhang J, Hizon R, Du T, Graham MR, Beniac DR, Booth TF, Kibsey P, Miller M, Gravel D, Mulvey MR, Canadian Nosocomial Infection Surveillance P. 2013. Characterization of a stable, metronidazole-resistant *Clostridium difficile* clinical isolate. PLoS One 8:e53757.
4. Cartman ST, Kelly ML, Heeg D, Heap JT, Minton NP. 2012. Precise manipulation of the *Clostridium difficile* chromosome reveals a lack of association between the tcdC genotype and toxin production. Appl Environ Microbiol 78:4683-90.
5. Heap JT, Pennington OJ, Cartman ST, Minton NP. 2009. A modular system for *Clostridium shuttle* plasmids. J Microbiol Methods 78:79-85.
6. Marreddy RKR, Wu X, Sapkota M, Prior AM, Jones JA, Sun D, Hevener KE, Hurdle JG. 2019. The Fatty Acid Synthesis Protein Enoyl-ACP Reductase II (FabK) is a Target for Narrow-Spectrum Antibacterials for *Clostridium difficile* Infection. ACS Infect Dis 5:208-217.