SUPPLEMENTAL INFORMATION

Excreted antibiotics may be key to emergence of increasingly efficient antibiotic resistance in food-animal production

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Table S1. Bacterial strains and plasmids used in this study.

Strains	Relevant genotype or phenotype[a]	Reference or source[b]
Escherichia coli		
DH10B	F- *mcrA* Δ(mrr-hsdRMS-*mcrBC*) 80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu)7697 galU galK λ- rpsL nupG	Thermo Fisher
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$	Str^r Cm^r; DH10B with pMMB207 vector and Tem-1 deletion (no-insert control)	
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::bla_{CMY-2}	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with bla_{CMY-2} and its native promoter (-574)	This study
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::$bla_{CTX-M-15}$	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with $bla_{CTX-M-15}$ and its native promoter (-506)	This study
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::bla_{KPC-3}	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with bla_{KPC-3} and its native promoter (-377)	This study
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::bla_{CMY-2}-Flagtagged	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with bla_{CMY-2}, its native promoter (-574) and flag-tag after signal peptide sequence	This study
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::$bla_{CTX-M-15}$-Flagtagged	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with $bla_{CTX-M-15}$, its native promoter (-506) and flag-tag after signal peptide sequence	This study
DH10B/ pMMB207Δ$bla_{\text{TEM-1}}$::bla_{KPC-3}-Flagtagged	Str^r Cm^r Amp^r; DH10B with pMMB207 vector containing Tem-1 deletion complemented with bla_{KPC-3}, its native promoter (-377) and flag-tag after signal peptide sequence	This study
Top10	F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K λ^- *rps*L(Str^R) *end*A1 *nup*G	Invitrogen
Top10/pET200::bla_{CMY-2}	Str^r Kan^r Amp^r; Top10 with the expression vector pET200 complemented with bla_{CMY-2}	This study
Top10/pET200::$bla_{CTX-M-15}$	Str^r Kan^r Amp^r; Top10 with the expression vector pET200 complemented with $bla_{CTX-M-15}$	This study
Top10/pET200::bla_{KPC-3}	Str^r Kan^r Amp^r; Top10 with the expression vector pET200 complemented with bla_{KPC-3}	This study
BL21 (DE3)	F^- *omp*T *hsd*S_B (r_B^-, m_B^-) *gal dcm* (DE3)	Invitrogen
BL21/pET200::bla_{CMY-2}	Kan^r Amp^r; Top10 with the expression vector pET200 complemented with bla_{CMY-2}	This study
BL21/pET200::$bla_{CTX-M-15}$	Kan^r Amp^r; Top10 with the expression vector pET200 complemented with $bla_{CTX-M-15}$	This study
BL21/pET200::bla_{KPC-3}	Kan^r Amp^r; Top10 with the expression vector pET200 complemented with bla_{KPC-3}	This study
Plasmids		
pMMB207	Cm^r, Amp^r; RSF1010 derivative, *IncQ lacI*^{q} Tac *ori*T	

pMMB207Δ*bla*$_{TEM-1}$	Cm^r; pMMB207 vector with Tem-1 cassette deletion	This study
pET200	Kan^r; T7 expression vector, N-terminal peptide containing the X-press epitope and the 6× His tag	Invitrogen
Wild-type bacterial strains		
AR-0044	*bla*$_{CTX-M-15}$-positive *E. coli*	CDC
AR-0081	*bla*$_{CMY-2}$-positive *K. pneumoniae*	CDC
AR-0114	*bla*$_{KPC-3}$-positive *E. coli*	CDC

[a] Amp^r, ampicillin resistant; Cm^r, Chloramphenicol resistant; Kan^r, kanamycin resistant; Str^r, streptomycin resistant; Nal^r, nalidixic acid resistant.
[b] E. coli isolates AR-0044, AR-0081 and AR-0114 were obtained from the CDC and FDA antibiotic resistance isolate bank.

Table S2. Primers used in this study.

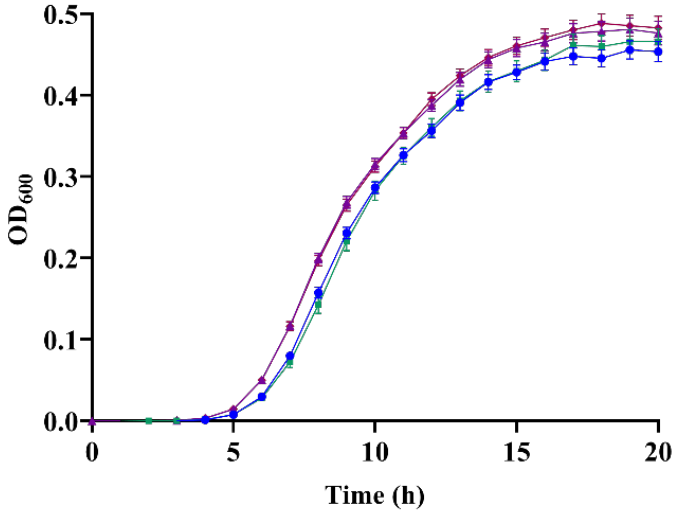
Primer	Sequence (5' – 3')[a]	Purpose	Reference
pMMB207 Tem-1 removal primers			
p207_InvPCR-F	CAGGCATCAACCCCGTCAGTAGCTGAACAG	Used for knocking out Tem-1 promoter and Tem-1 gene in pMMB207	This study
p207_InvPCR-R	GACGGGGTGTGATGCCTGGCAGTTTATGGCG	Used for knocking out Tem-1 promoter and Tem-1 gene in pMMB207	This study
p207_Tem-1_F	GTATCCGCTCATGAGACAATAACCCTGATA	Used to confirm the deletion of Tem-1 cassette	This study
p207_Tem-1_R	GTGCACCCAACTGATCTTCAGCATC	Used to confirm the deletion of Tem-1 cassette	This study
Cloning of AR genes into pMMB207ΔTem-1 primers			
Prom+_CTXM&CMY-2	ATTCGGAGCTCTGGGTCATCTCTTGCTAAAGTCA	Used for cloning of *bla*$_{CMY-2}$ and *bla*$_{CTX-M-15}$ with promoter region (-574 and -506 respectively) into pMMB207ΔTem-1	This study
CMY-2_R	ATTCGGGATCCTTATTGCAGCTTTTCAAGAATGCG	Used for cloning of *bla*$_{CMY-2}$ into pMMB207ΔTem-1 (includes TTA stop codon)	This study
CTXM-15_R	ATTCGAAGCTTTTACAAACCGTCGGTGACGATTTTAG	Used for cloning of *bla*$_{CTX-M-15}$ into pMMB207ΔTem-1 (includes TTA stop codon)	This study
Prom_KPC-3_F	ATTCGGAGCTCGTCAGTATTACTTTGGTGATTCAG	Used for cloning of *bla*$_{KPC-3}$ with promoter region (-377) into pMMB207ΔTem-1	This study
KPC-3_R	ATTCGAAGCTTTTACTGCCCGTTGACGC	Used for cloning of *bla*$_{KPC-3}$ into pMMB207ΔTem-1 (includes TTA stop codon)	This study
M13R49	GAGCGGATAACAATTTCACACAGG	Used to verify the insertion of *bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$ and *bla*$_{KPC-3}$ genes into pMMB207ΔTem-1	Eurofins
TrcHis-R	CTTCTGCGTTCTGATTTAATCTG	Used to verify the insertion of *bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$ and *bla*$_{KPC-3}$ genes into pMMB207ΔTem-1	Eurofins

Protein expression constructs			
P-1_Flag-CMY-2-R	TGTTGTTCTGTTTTTGCCTTATCGTCGTCATCCTTGTAATCGGCAGCAAATGTG	Used for insertion of Flag-tag after signal peptide of *bla*$_{CMY-2}$	This study
P-2_CMY-2-F	CTCTTTCTCCACATTTGCTGCCGATTACAAGGATGACGACGATAAGGCAAAAACAG	Used for insertion of Flag-tag after signal peptide of *bla*$_{CMY-2}$	This study
P-1_Flag-CTXM-15-R	CTGTACGTCCGCCGTCTTATCGTCGTCATCCTTGTAATCTTGCGCATAC	Used for insertion of Flag-tag after signal peptide of *bla*$_{CTX-M-15}$	This study
P-2_CTXM-15-F	GCTGTATGCGCAAGATTACAAGGATGACGACGATAAGACGGCGGAC	Used for insertion of Flag-tag after signal peptide of *bla*$_{CTX-M-15}$	This study
P1_KPC3_Flag-R	GACGAGGTTGGTCAGCTTATCGTCGTCATCCTTGTAATCCGCGGTGGCAGAAAAG	Used for insertion of Flag-tag after signal peptide of *bla*$_{KPC-3}$	This study
P2_KPC3_Flag_F	GCTTTTCTGCCACCGCGGATTACAAGGATGACGACGATAAGCTGACCAACCTCGTC	Used for insertion of Flag-tag after signal peptide of *bla*$_{KPC-3}$	This study
Enzyme kinetics constructs			
pET200-CTX-M-15-F	CACCATGGTTAAAAAATCACTGCGCCAGTT	Used for the cloning of blunt-end *bla*$_{CTX-M-15}$ into pET200 vector for protein expression and purification (paired with primer CTXM-15_R)	This study
pET200-CMY-2-F	CACCATGATGAAAAAATCGTTATGCTGCGCTCTG	Used for the cloning of blunt-end *bla*$_{CMY-2}$ into pET200 vector for protein expression and purification (paired with primer CMY-2_R)	This study
pET200-KPC-3-F	CACCATGTCACTGTATCGCCGTCTAGTT	Used for the cloning of blunt-end *bla*$_{KPC-3}$ into pET200 vector for protein expression and purification (paired with primer KPC-3_R)	This study
T7	TAATACGACTCACTATAGGG	Used to verify the insertion of *bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$, and *bla*$_{KPC-3}$ into pET200 vector	Eurofins
T7-term	CTAGTTATTGCTCAGCGGT	Used to verify the insertion of *bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$, and *bla*$_{KPC-3}$ into pET200 vector	Eurofins
qPCR primers			
qPCR_CMY-2_F	CACCCAGTCACGCAGCAAACG	Used for the identification of *bla*$_{CMY-2}$ containing clones in the competition assays	This study
qPCR_CMY-2_R	GATAGCATCGCCGCCCAACAC	Used for the identification of *bla*$_{CMY-2}$ containing clones in the competition assays	This study

qPCR_CTX-M-15_F	GACTGCCTGCTTCCTGGGTTG	Used for the identification of *bla*$_{CTX-M-15}$ containing clones in the competition assays	This study
qPCR_CTX-M-15_R	GGTTGAGGCTGGGTGAAGTAAGTG	Used for the identification of *bla*$_{CTX-M-15}$ containing clones in the competition assays	This study
qPCR_KPC-3_F	CTGACAACAGGCATGACGGT	Used for the identification of *bla*$_{KPC-3}$ containing clones in the competition assays	This study
qPCR_KPC-3_R	GATAGAGCGCATGAAGGCCG	Used for the identification of *bla*$_{KPC-3}$ containing clones in the competition assays	This study

[a]Restriction enzyme sites are underline.

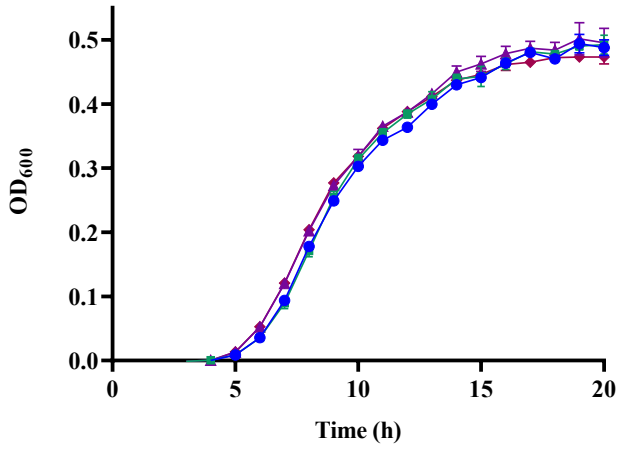
Fig. S1. Growth of *E. coli* cultures without antibiotic. Growth curves for CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**). Average (+/- SEM) optical density for each time point was based on 15 experiments for which **no antibiotic** was added to the media. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.73 [a]	4.74 [a]	5.17 [b]	5.2 [b]
(SEM)	(0.09)	(0.12)	(0.1)	(0.1)

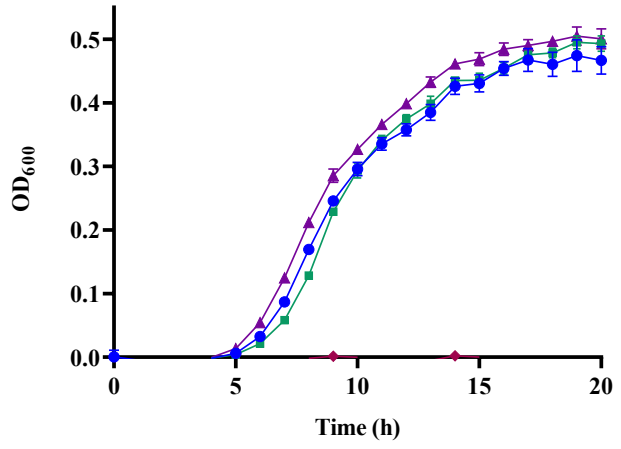
Fig. S2. Growth of *E. coli* cultures when exposed to ampicillin. Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) in the presence of 0, 8, 16, 32, 64, 128, 256, 512, 1,000, 1,500, 2,000, 2,500 or 3,000 µg/ml of **ampicillin**. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No antibiotic



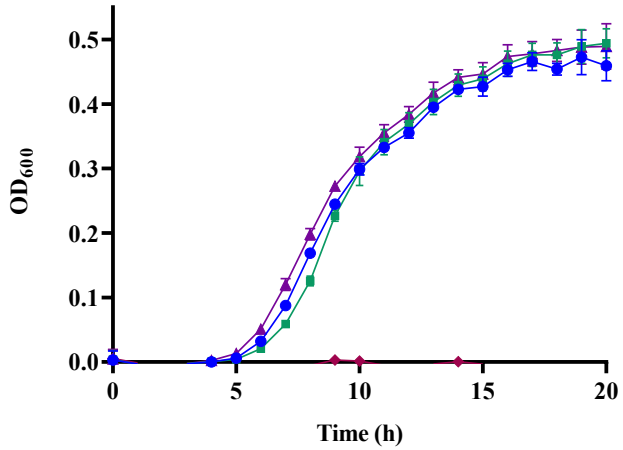
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	5.01 [a]	5.07 [a]	5.27 [a]	5.16 [a]
(SEM)	(0.03)	(0.04)	(0.05)	(0.03)

B) 8 µg/ml ampicillin



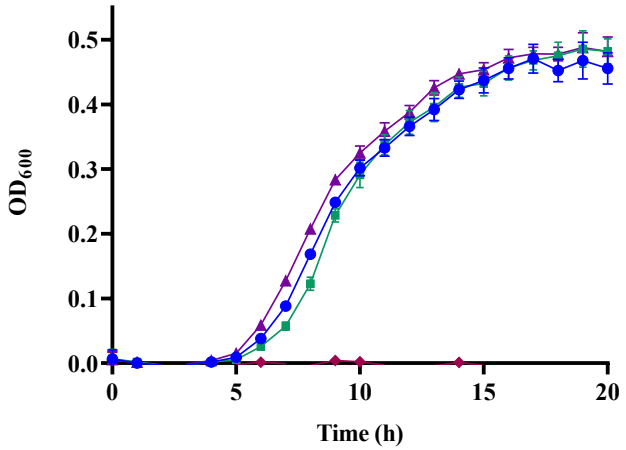
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.87 [a]	4.87 [a]	5.39 [b]	0.05 [c]
(SEM)	(0.06)	(0.04)	(0.04)	(0.01)

C) 16 µg/ml ampicillin



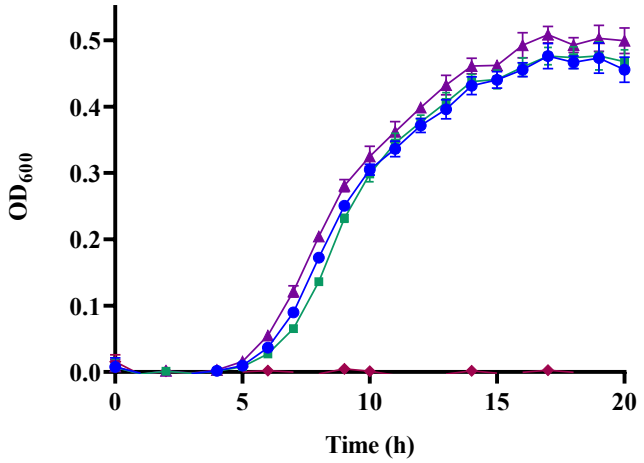
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.86 [a]	4.88 [a]	5.2 [a]	0.05 [b]
(SEM)	(0.06)	(0.08)	(0.08)	(0.01)

D) 32 µg/ml ampicillin



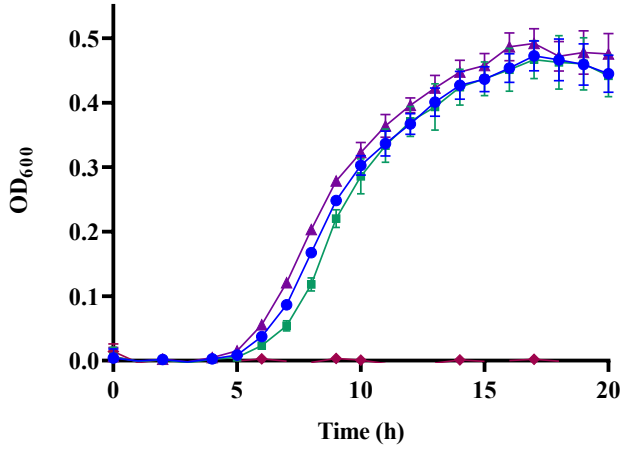
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.89 [a]	4.83 [a]	5.26 [a]	0.04 [b]
(SEM)	(0.07)	(0.08)	(0.06)	(0.01)

E) 64 µg/ml ampicillin



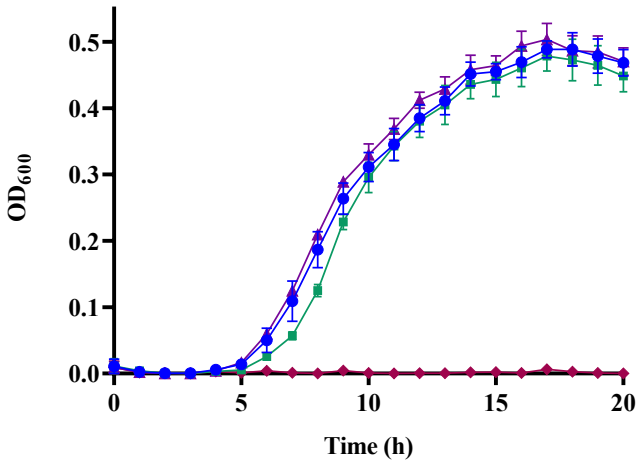
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.95 [a]	4.9 [a]	5.37 [a]	0.04 [b]
(SEM)	(0.06)	(0.06)	(0.06)	(0.01)

F) 128 µg/ml ampicillin



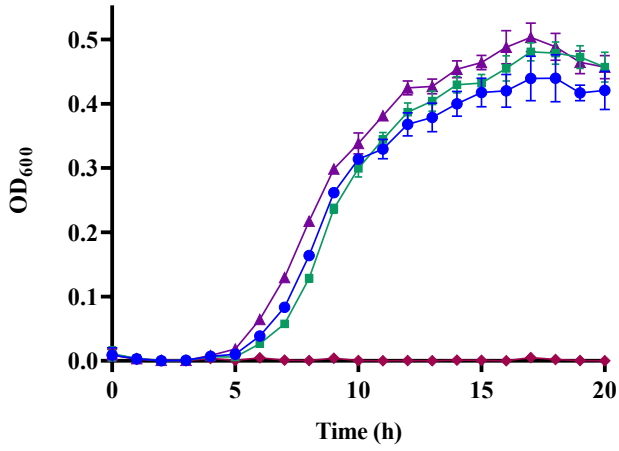
	CMY-2	CTX-M-15	KPC-3	Control
	4.90 [a]	4.73 [a]	5.26 [a]	0.04 [b]
	(0.09)	(0.13)	(0.09)	(0.01)

G) 256 µg/ml ampicillin



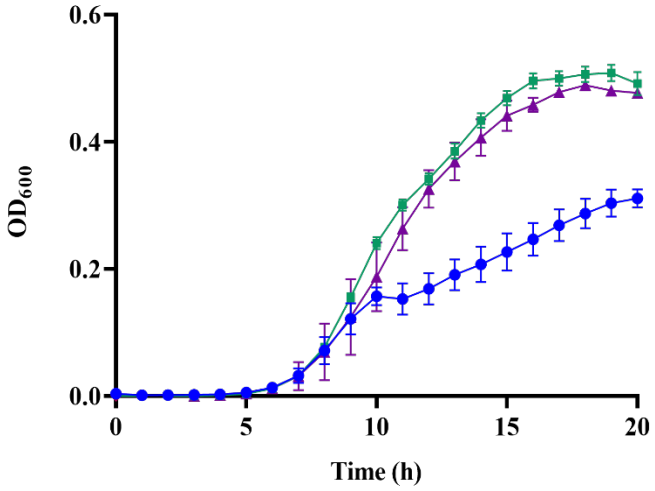
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	5.16 [a]	4.86 [a]	5.38 [a]	0.04 [b]
(SEM)	(0.1)	(0.11)	(0.08)	(0.01)

H) 512 µg/ml ampicillin



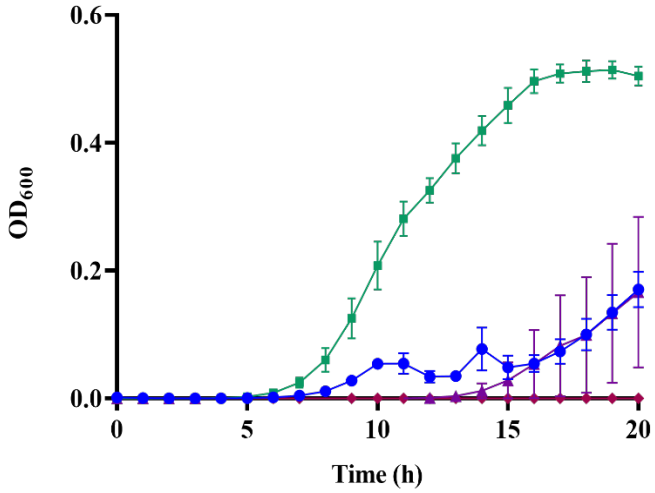
	CMY-2	CTX-M-15	KPC-3	Control
	4.71 [a]	4.88 [a]	5.41 [a]	0.03 [b]
	(0.09)	(0.06)	(0.07)	(0.01)

I) 1,000 µg/ml ampicillin



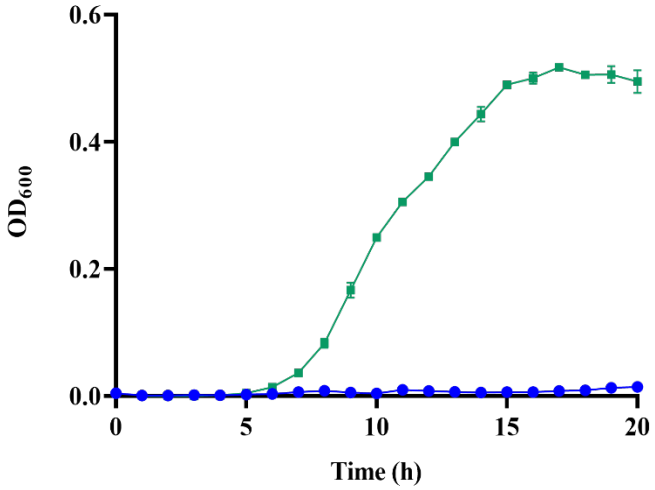
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	2.62[a]	4.71[b]	4.38[b]	0.01 [c]
(SEM)	(0.10)	(0.05)	(0.14)	(0.003)

J) 1,500 µg/ml ampicillin



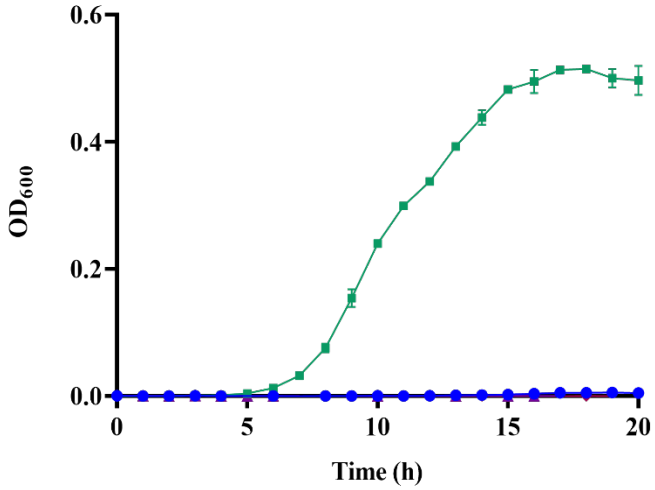
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	0.80[a]	4.57[b]	0.5 [c]	0.003 [c]
(SEM)	(0.08)	(0.1)	(0.24)	(0.001)

K) 2,000 µg/ml ampicillin



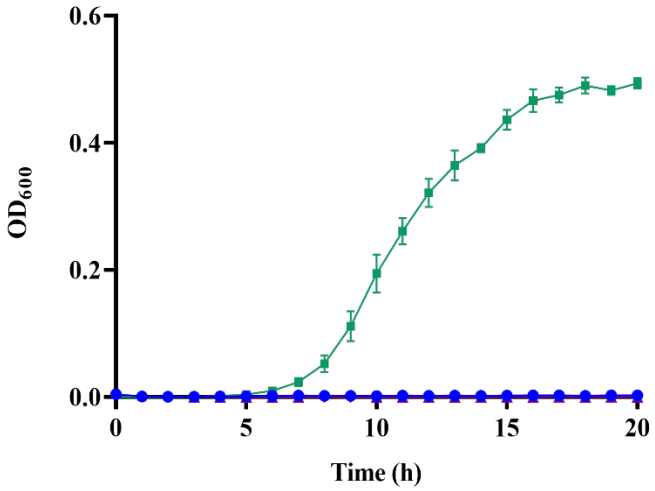
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	0.12[a]	4.82[b]	0.02[a]	0.01[a]
(SEM)	(0.02)	(0.04)	(0.002)	(0.002)

L) 2,500 µg/ml ampicillin



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	0.03[a]	4.74[b]	0.01[a]	0.01[a]
(SEM)	(0.01)	(0.05)	(0.002)	(0.002)

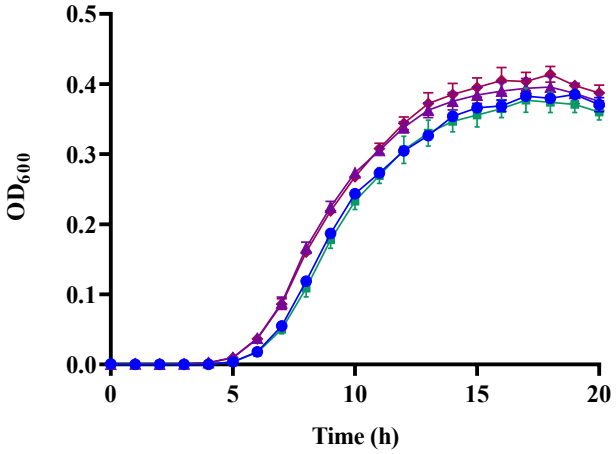
M) 3,000 µg/ml ampicillin



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	0.04[a]	4.33[b]	0.01[a]	0.01[a]
(SEM)	(0.004)	(0.08)	(0.003)	(0.003)

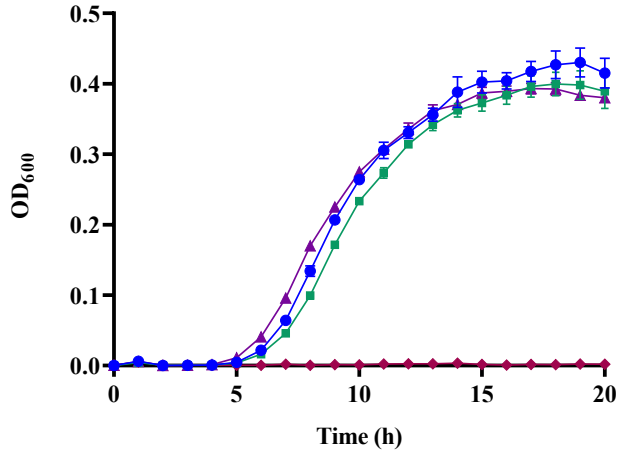
Fig. S3. Growth of *E. coli* cultures when exposed to ceftiofur. Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) in the presence of 0, 4, 8, 16, 32, 64, 128, or 256 µg/ml of **ceftiofur**. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No antibiotic



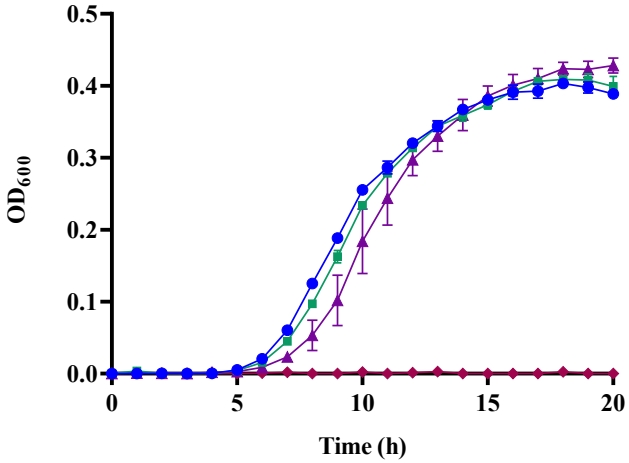
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	3.95 [a]	3.87 [a]	4.32 [a]	4.4 [a]
(SEM)	(0.02)	(0.06)	(0.05)	(0.05)

B) 4 µg/ml ceftiofur



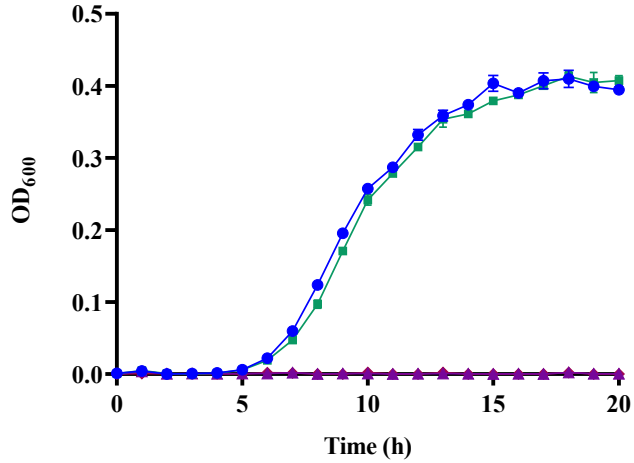
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.37[a]	4.01[a]	4.34[a]	0.03 [b]
(SEM)	(0.06)	(0.05)	(0.0)	(0.01)

C) 8 µg/ml ceftiofur



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.13[a]	4.01[a]	3.87[a]	0.01 [b]
(SEM)	(0.03)	(0.03)	(0.11)	(0.01)

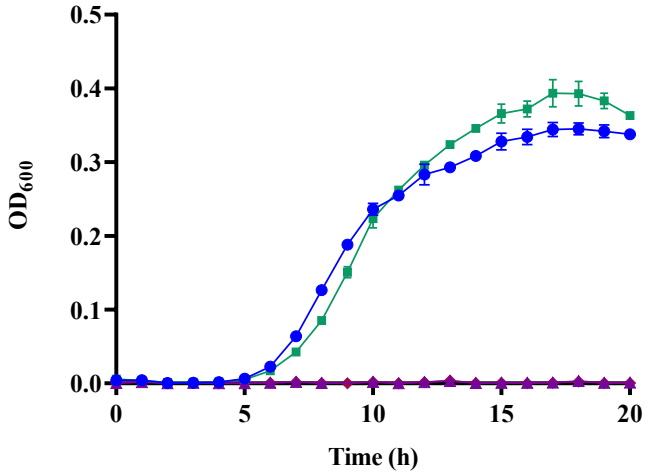
D) 16 µg/ml ceftiofur



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.23[a]	4.09[a]	0.02 [b]	0.01 [b]
(SEM)	(0.03)	(0.03)	(0.01)	(0.01)

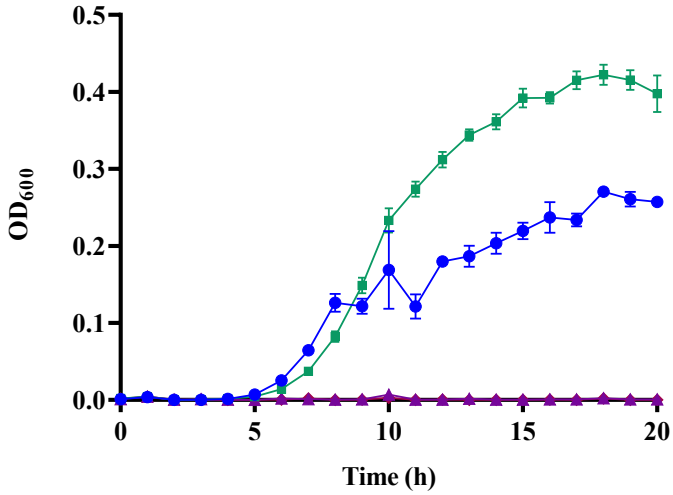
bla_{CMY-2} $bla_{CTX-M-15}$ bla_{KPC-3} pMMB207Δbla_{Tem-1} (control)

E) 32 µg/ml ceftiofur



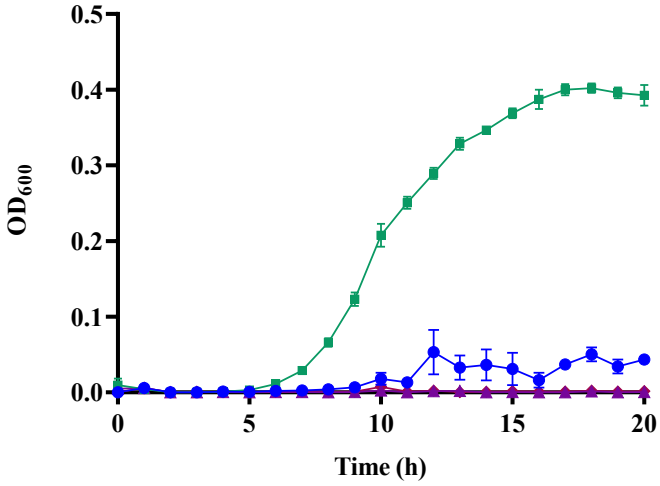
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	3.65[a] (0.04)	3.85[a] (0.05)	0.02 [b] (0.01)	0.02 [b] (0.01)

F) 64 µg/ml ceftiofur



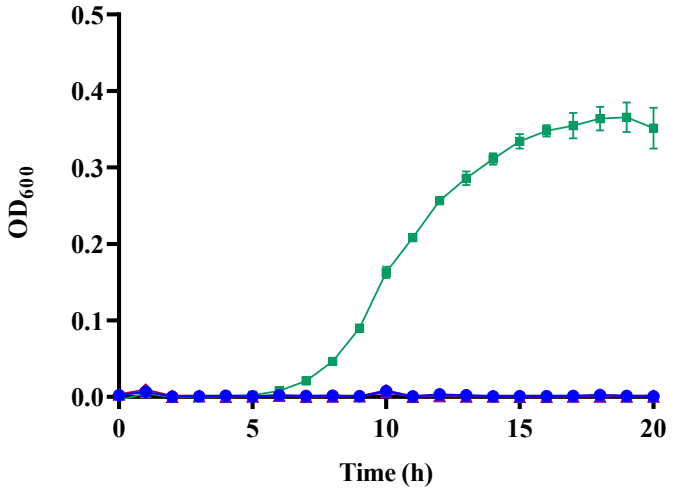
	CMY-2	CTX-M-15	KPC-3	Control
	2.56[a] (0.08)	4.05 [b] (0.05)	0.03[c] (0.01)	0.02[c] (0.01)

G) 128 µg/ml ceftiofur



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	0.37[a] (0.06)	3.82 [b] (0.04)	0.02 [c] (0.01)	0.03[c] (0.01)

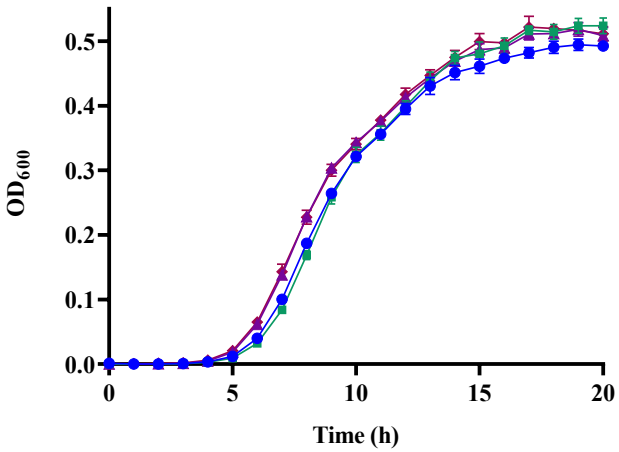
H) 256 µg/ml ceftiofur



	CMY-2	CTX-M-15	KPC-3	Control
	0.04[a] (0.01)	3.34 [b] (0.05)	0.02 [a] (0.01)	0.04 [a] (0.01)

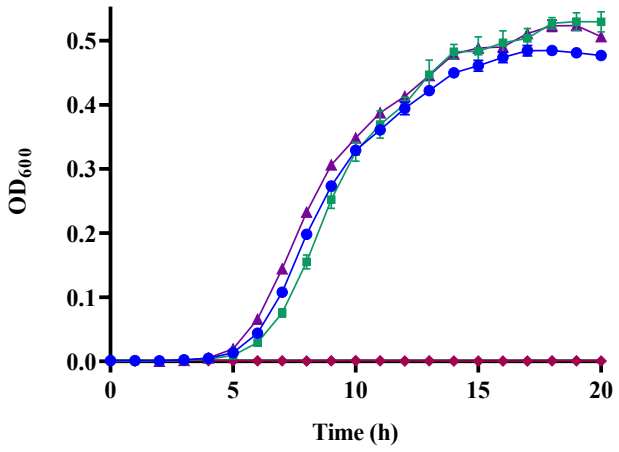
Fig. S4. Growth of *E. coli* cultures when exposed to desfuroylceftiofur (DFC). Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) in the presence of 0, 4, 8, 16, 32, 64, 128, or 256 µg/ml of **DFC**. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No antibiotic



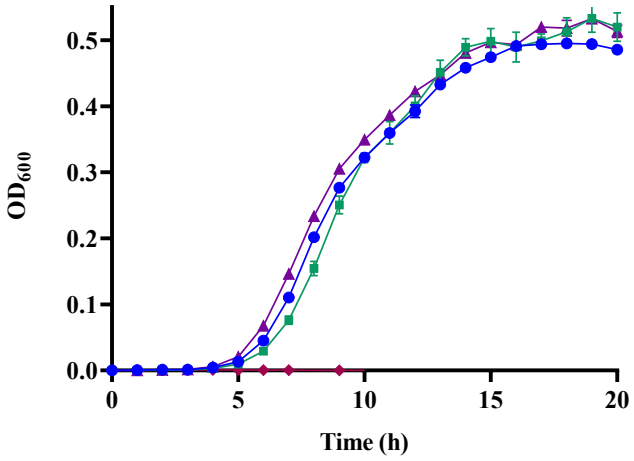
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.21[a] (0.04)	5.34[a] (0.05)	5.57[a] (0.03)	5.63[a] (0.05)

B) 4 µg/ml DFC



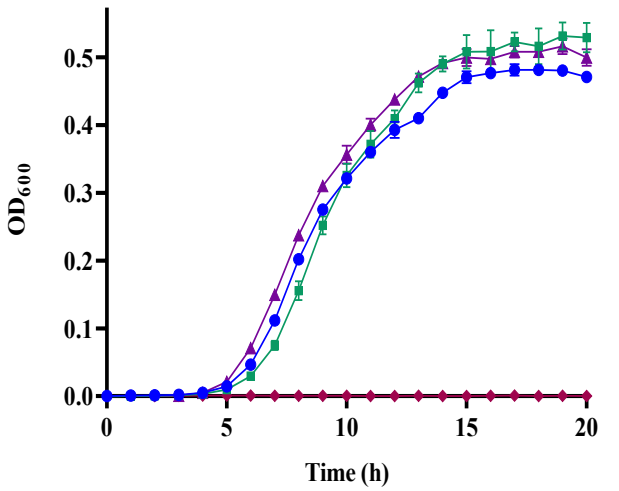
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.23[a] (0.03)	5.36[a] (0.07)	5.64[a] (0.03)	0.01[b] (0.002)

C) 8 µg/ml DFC

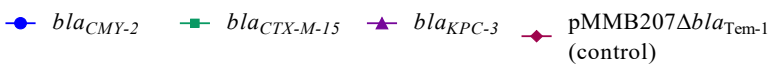


	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.31[a] (0.03)	5.34[a] (0.07)	5.67[a] (0.03)	0.01[b] (0.002)

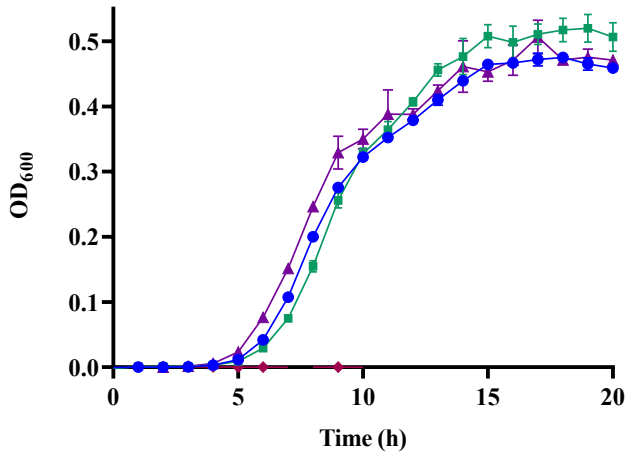
D) 16 µg/ml DFC



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.22[a] (0.03)	5.44[a] (0.08)	5.73[a] (0.04)	0.01[b] (0.001)

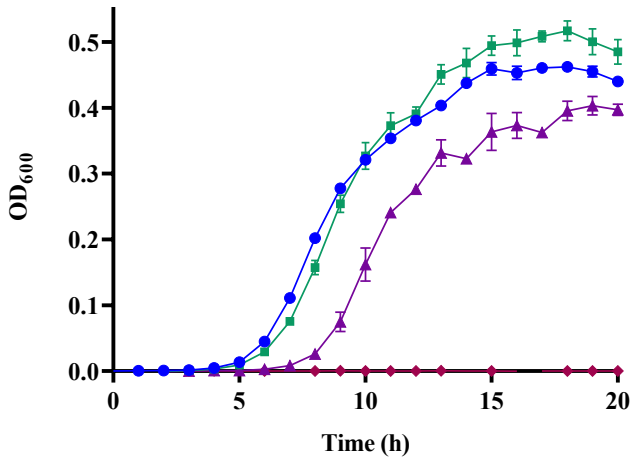


E) 32 µg/ml DFC



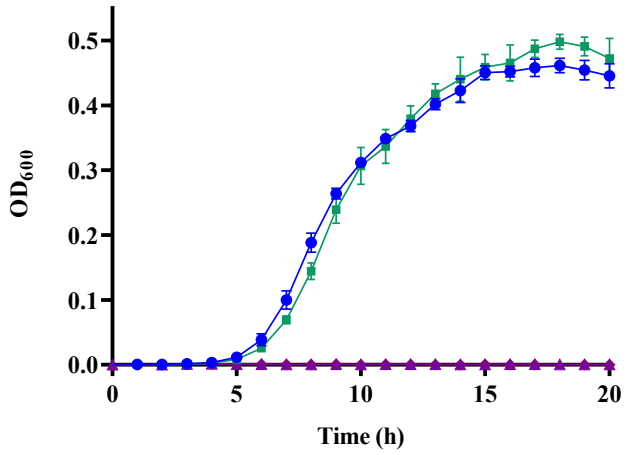
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.12[a] (0.03)	5.37[a] (0.07)	5.46[a] (0.09)	0.01[b] (0.002)

F) 64 µg/ml DFC



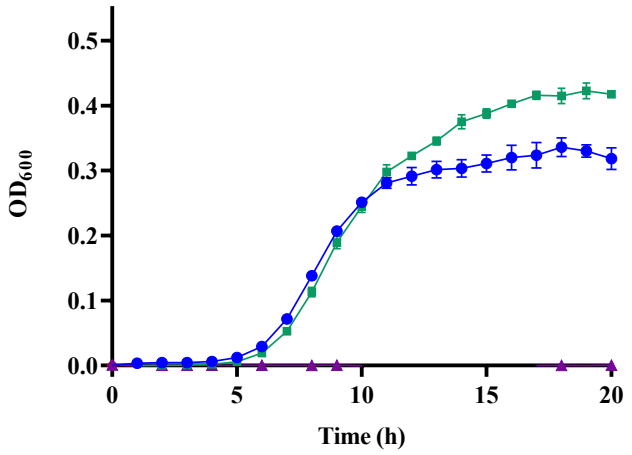
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	5.06[a] (0.03)	5.30[a] (0.07)	3.55[b] (0.07)	0.01[c] (0.002)

G) 128 µg/ml DFC



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.96[a] (0.03)	5.01[a] (0.06)	0.01[b] (0.001)	0.01[b] (0.001)

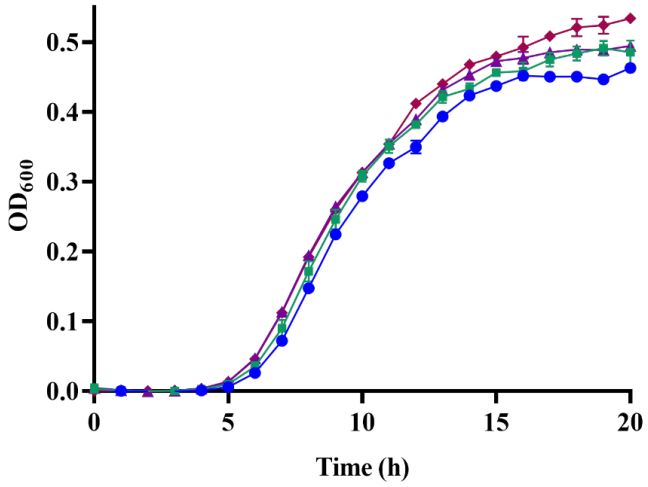
H) 256 µg/ml DFC



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	3.69[a] (0.06)	4.22[b] (0.04)	0.01[c] (0.004)	0.03[c] (0.01)

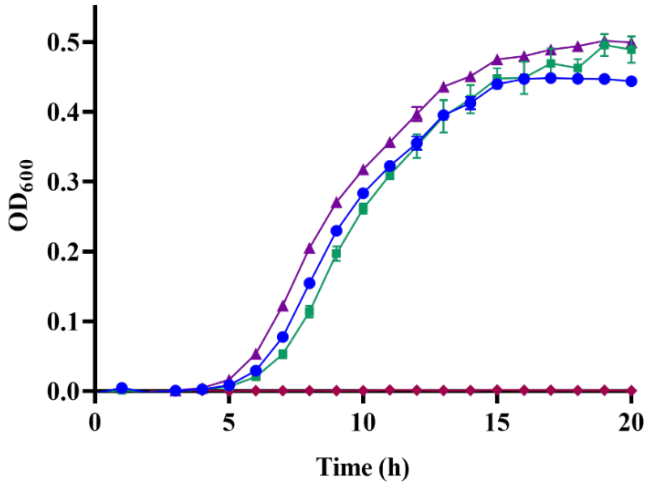
Figure S5. Growth of *E. coli* cultures when exposed to DFC-cysteine. Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) in the presence of 0, 4, 8, 16, 32, 64, 128, or 256 µg/ml of **DFC-cysteine**. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No antibiotic



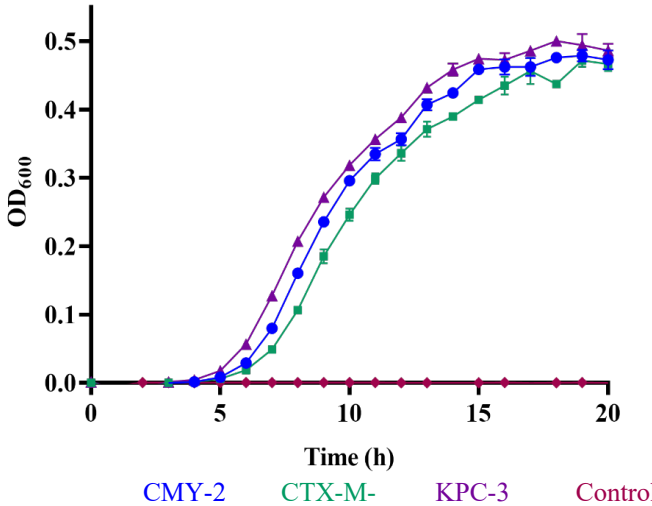
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.72[a] (0.03)	5.06[b] (0.05)	5.24[b] (0.02)	5.41[b] (0.04)

B) 4 µg/ml DFC-cysteine



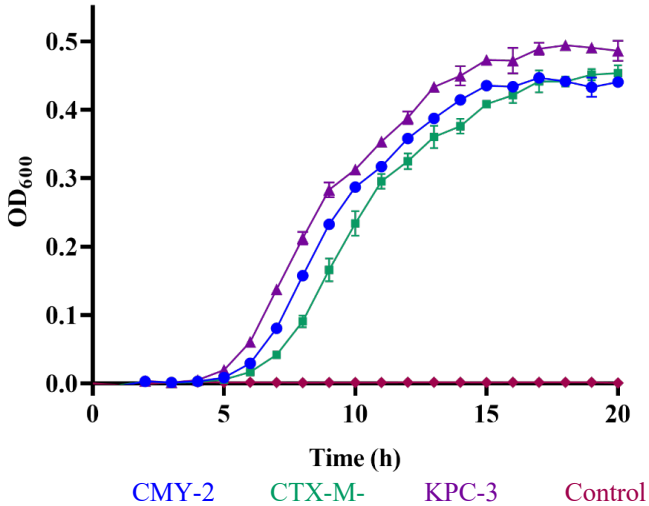
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.73[a] (0.02)	4.70[a] (0.07)	5.33[b] (0.03)	0.02[c] (0.003)

C) 8 µg/ml DFC-cysteine



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.92[a] (0.04)	4.47[b] (0.1)	5.33[c] (0.04)	0.01[d] (0.01)

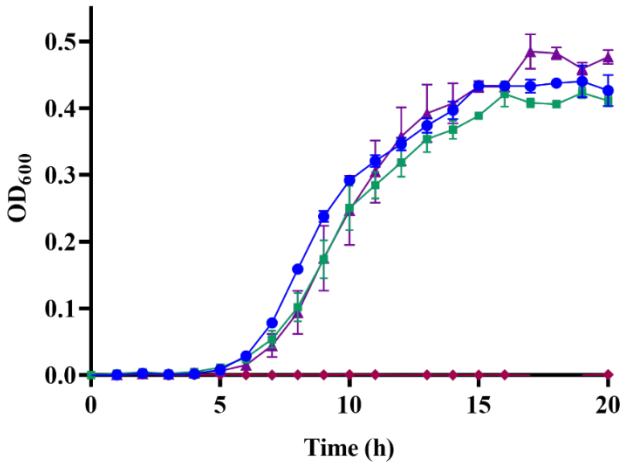
D) 16 µg/ml DFC-cysteine



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.7[a] (0.03)	4.31[b] (0.05)	5.33[c] (0.04)	0.03[d] (0.01)

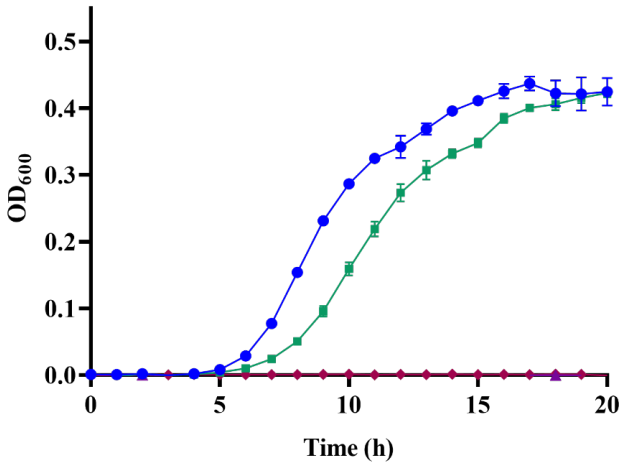
bla$_{CMY-2}$ *bla*$_{CTX-M-15}$ *bla*$_{KPC-3}$ pMMB207Δ*bla*$_{Tem-1}$ (control)

E 32 µg/ml DFC-cysteine



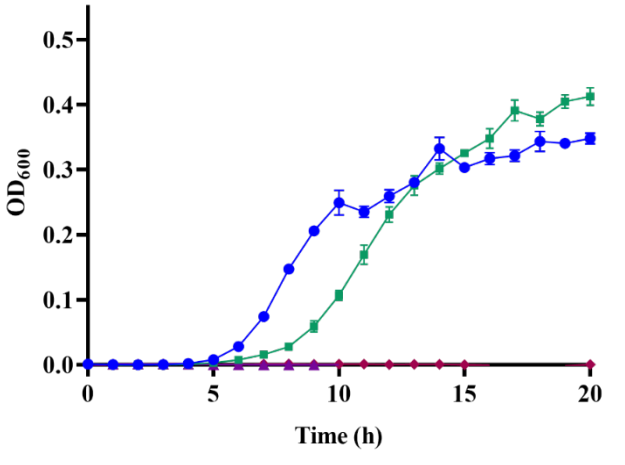
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.64[a]	4.21[a]	4.58[a]	0.01[b]
(SEM)	(0.1)	(0.1)	(0.15)	(0.003)

F) 64 µg/ml DFC-cysteine



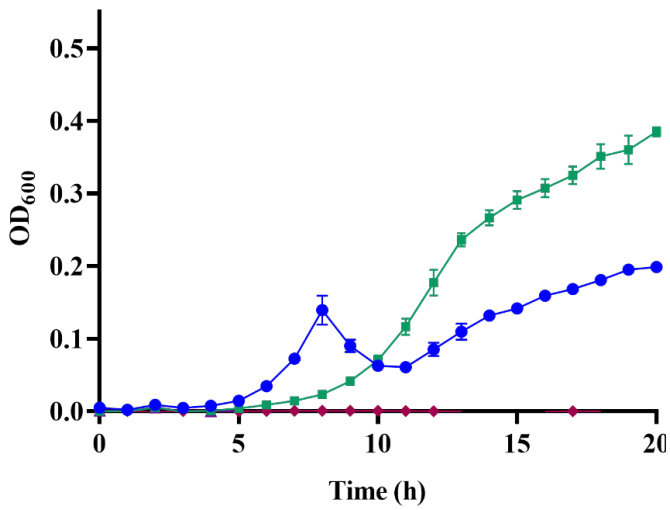
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.55[a]	3.65[b]	0.01[c]	0.02[c]
(SEM)	(0.1)	(0.04)	(0.003)	(0.01)

G) 128 µg/ml DFC-cysteine



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	3.62[a]	3.26[b]	0.02[c]	0.02[c]
(SEM)	(0.1)	(0.1)	(0.004)	(0.01)

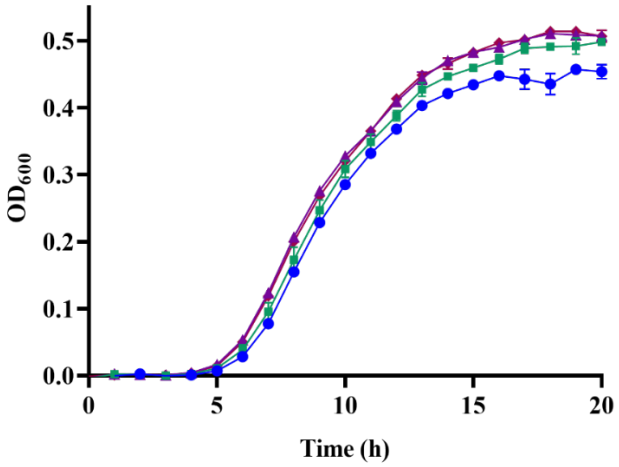
H) 256 µg/ml DFC-cysteine



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	1.77[a]	2.79[b]	0.03[c]	0.01[c]
(SEM)	(0.04)	(0.1)	(0.01)	(0.01)

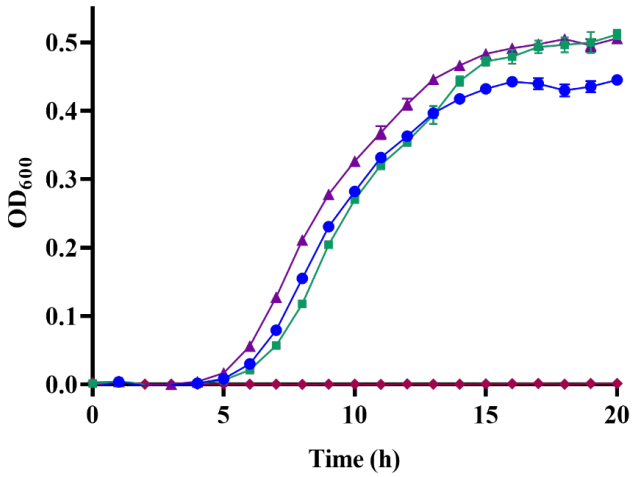
Figure S6. Growth of *E. coli* cultures when exposed to DFC-dimer. Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) in the presence of 0, 4, 8, 16, 32, 64, 128, or 256 µg/ml of **DFC-dimer**. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No antibiotic



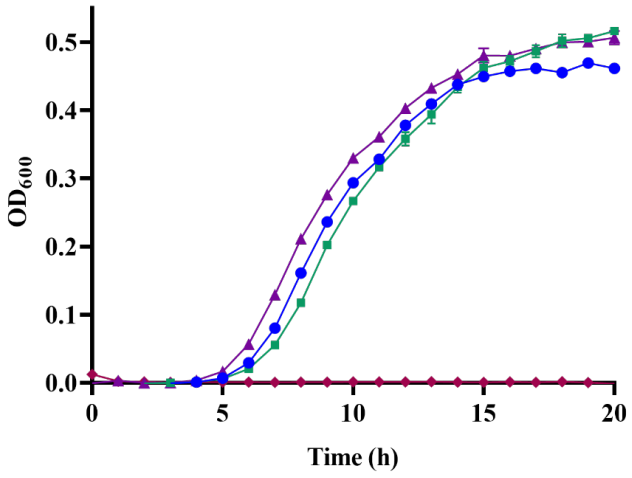
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.76[a]	5.15[b]	5.46[b]	5.44[b]
(SEM)	(0.04)	(0.10)	(0.02)	(0.02)

B) 4 µg/ml DFC-dimer



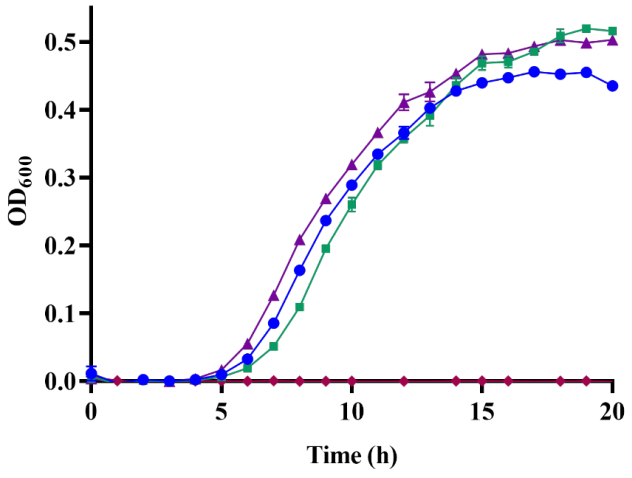
	CMY-2	CTX-M-15	KPC-3	Control
	4.70[a]	4.9[b]	5.44[c]	0.02[d]
	(0.03)	(0.04)	(0.03)	(0.01)

C) 8 µg/ml DFC-dimer



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	4.89[a]	4.87[a]	5.38[b]	0.03[c]
(SEM)	(0.02)	(0.03)	(0.03)	(0.01)

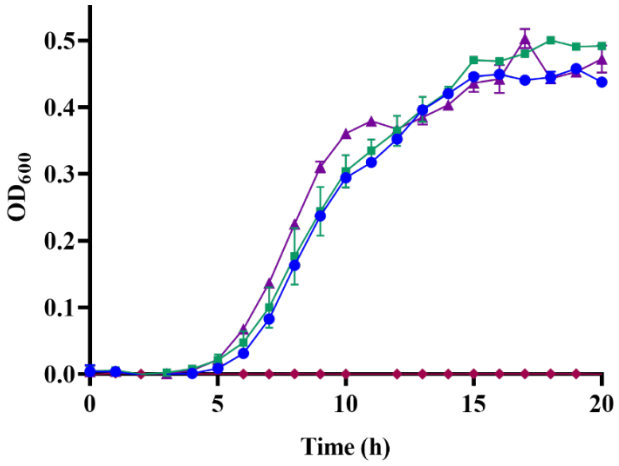
D) 16 µg/ml DFC-dimer



	CMY-2	CTX-M-15	KPC-3	Control
	4.83[a]	4.87[a]	5.37[b]	0.01[c]
	(0.03)	(0.04)	(0.03)	(0.01)

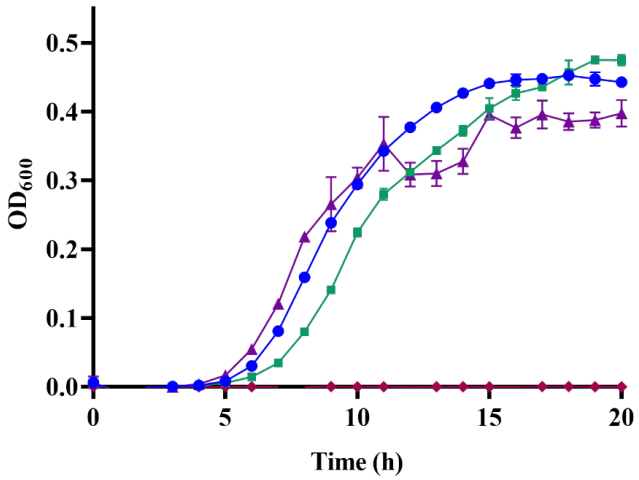
bla_{CMY-2} $bla_{CTX-M-15}$ bla_{KPC-3} pMMB207Δbla_{Tem-1} (control)

E) 32 µg/ml DFC-dimer



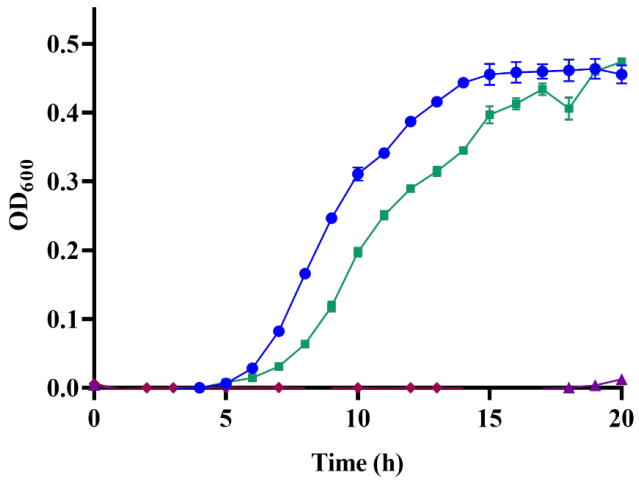
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.77[a] (0.02)	5.1[a] (0.1)	5.18[a] (0.05)	0.01[b] (0.003)

F) 64 µg/ml DFC-dimer



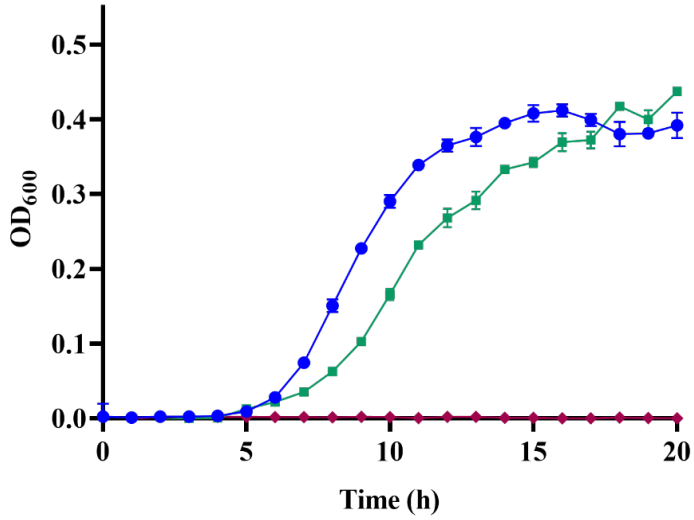
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.84[a] (0.03)	4.26[b] (0.04)	4.43[c] (0.1)	0.01[d] (0.01)

G) 128 µg/ml DFC-dimer



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.97[a] (0.1)	3.99[b] (0.03)	0.03[c] (0.01)	0.02[c] (0.01)

H) 256 µg/ml DFC-dimer



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.44[a] (0.1)	3.66[b] (0.04)	0.03[c] (0.01)	0.03[c] (0.02)

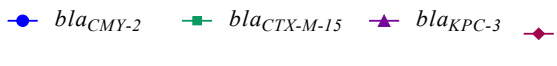


Fig. S7. SDS-PAGE analysis for CTX-M-15, KPC-3, and CMY-2 after HPLC purification. Coomassie stained SDS-PAGE gel showing a mass standard (M), CTX-M-15 (31 kDa), KPC-3 (32 kDa) and CMY-2 (37 kDa) protein bands. These preparations were used to estimate catalytic efficiency for each enzyme.

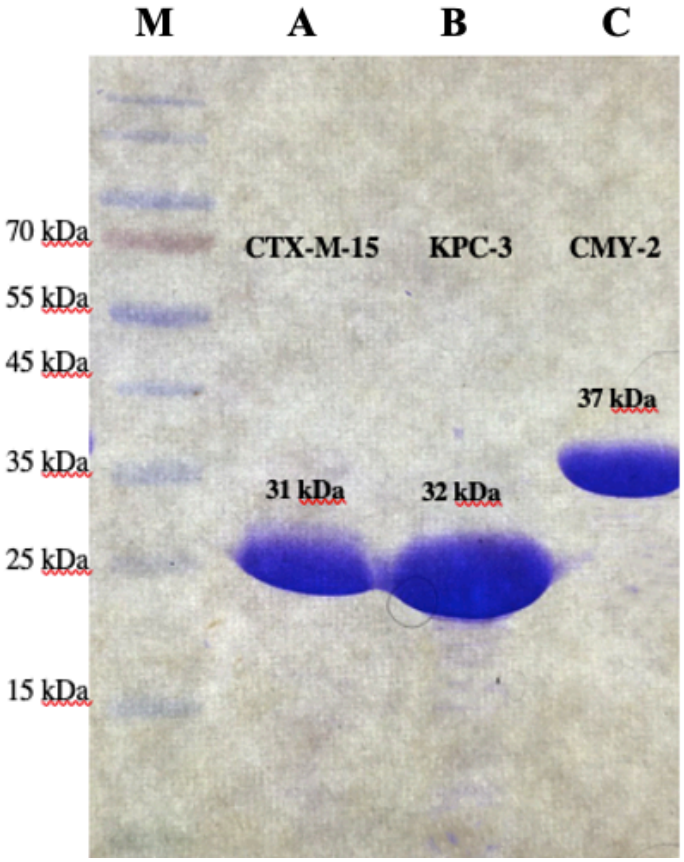
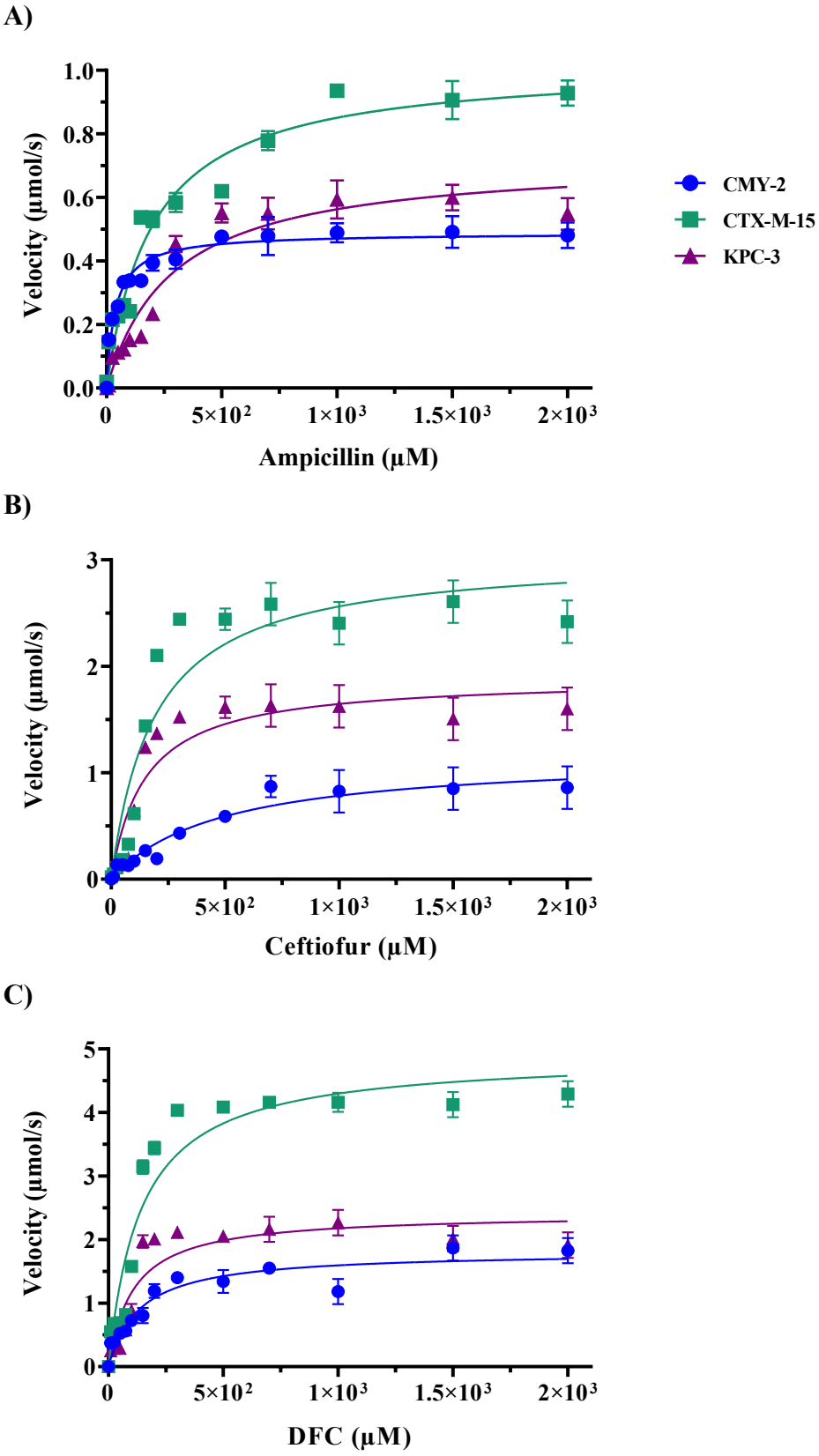
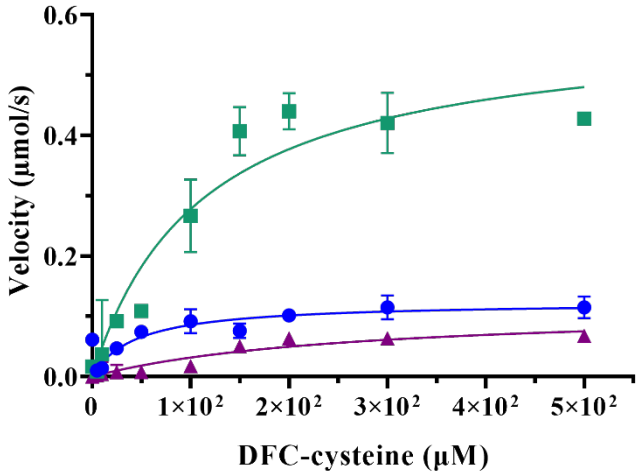


Fig. S8. Kinetic assays of CTX-M-15, CMY-2, and KPC-3 against (A) ampicillin, (B) ceftiofur (C) desfuroylceftiofur (DFC), (C) DFC-cysteine, (D) DFC-dimer and (E) DFC-dimer. Velocity plots are used in the calculation of steady-state kinetic parameters (Table 1). GraphPad Prism (San Diego, CA) was used to draw the plots. Data points represent the average of three independent replicates +/- SEM. See Table 1, Fig. S7 and the methods section for additional information.



D)


0.6
0.4
0.2
0.0
Velocity (µmol/s)
0
1×10²
2×10²
3×10²
4×10²
5×10²
DFC-cysteine (µM)

E)

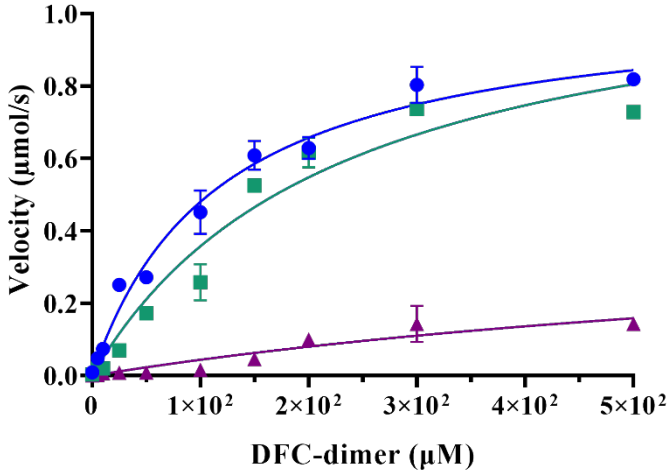
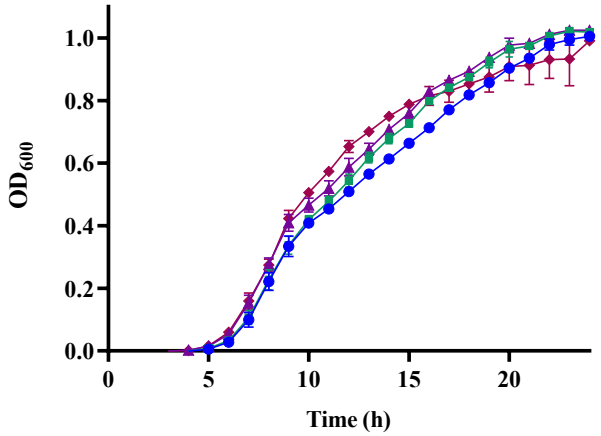

1.0
0.8
0.6
0.4
0.2
0.0
Velocity (µmol/s)
0
1×10²
2×10²
3×10²
4×10²
5×10²
DFC-dimer (µM)

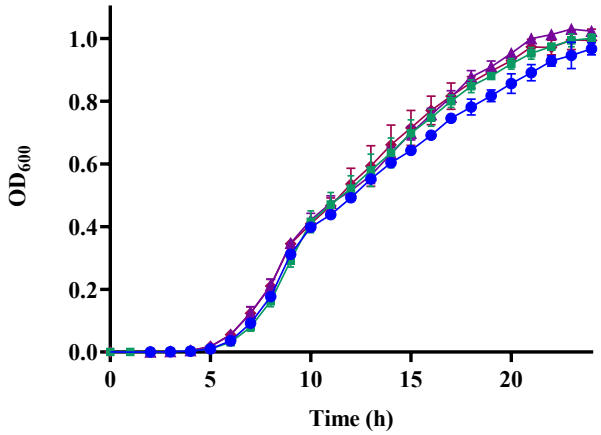
Fig. S9. *E. coli* cultures with ceftiofur added at 5 or 8 hours post inoculation. Average growth curves (3 independent replicates, +/- SEM) for the CTX-M-15- (**green square**), CMY-2- (**blue circle**), and KPC-3- (**purple triangle**) producing strains of *E. coli* and for the plasmid-only negative control strain (**red diamond**) with no antibiotic for the first five hours (left column) or for the first 8 hours (right column) before adding 4, 8, 16, 32, 64, 128, or 256 µg/ml ceftiofur. The tables below each figure present average area under the curve (AUC) with the standard error of the mean (SEM). Subscripts represent statistically nonsignificant grouping according to one-way ANOVA and Tukey's multiple comparisons test, (P<0.05).

A) No ceftiofur added at 5 hours



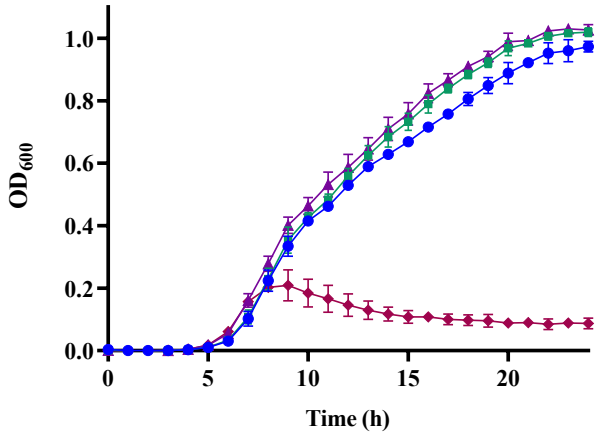
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	11.38[a]	12.09[a]	12.63[a]	12.46[a]
(SEM)	(0.1)	(0.1)	(0.1)	(0.2)

B) No ceftiofur added at 8 hours



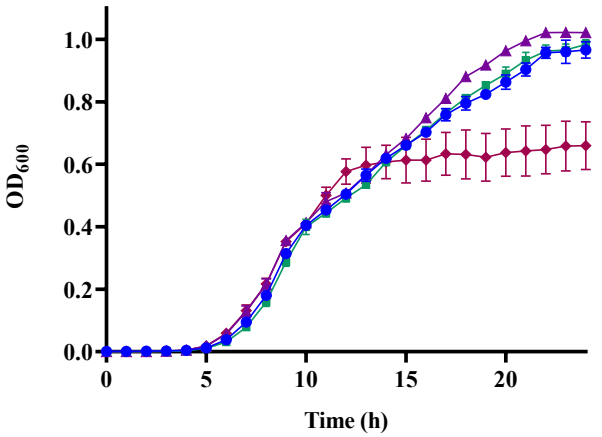
	CMY-2	CTX-M-15	KPC-3	Control
	10.90[a]	11.52[a]	11.93[a]	11.85[a]
	(0.1)	(0.15)	(0.09)	(0.19)

C) 4 µg/ml ceftiofur added at 5 hours



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	11.34[a]	12.17[a]	12.7[a]	2.3[b]
(SEM)	(0.11)	(0.12)	(0.14)	(0.14)

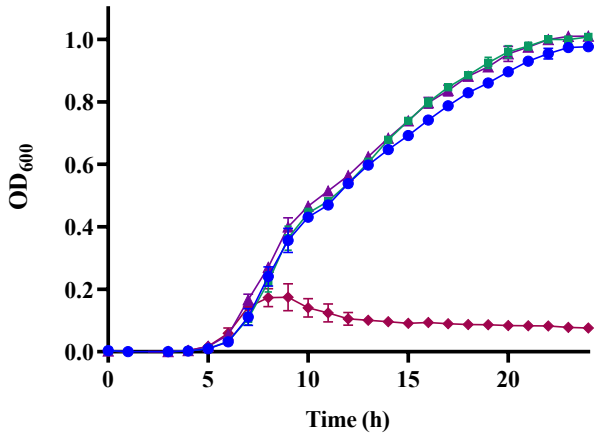
D) 4 µg/ml ceftiofur added at 8 hours



	CMY-2	CTX-M-15	KPC-3	Control
	11.1[a]	11.1[a]	11.93[a]	9.51[b]
	(0.1)	(0.08)	(0.07)	(0.31)

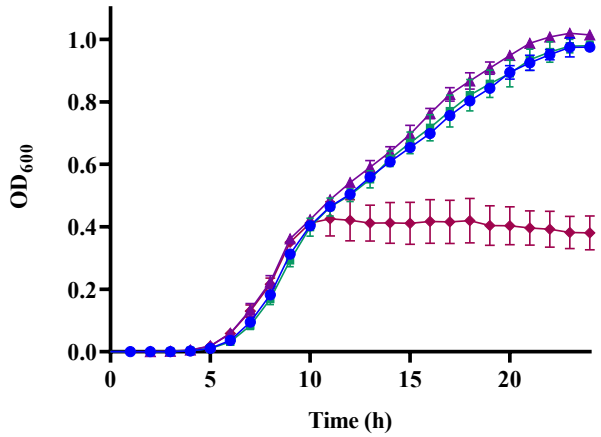
bla$_{CMY-2}$ *bla*$_{CTX-M-15}$ *bla*$_{KPC-3}$ pMMB207Δ*bla*$_{Tem-1}$ (control)

E) 8 µg/ml ceftiofur added at 5 hours



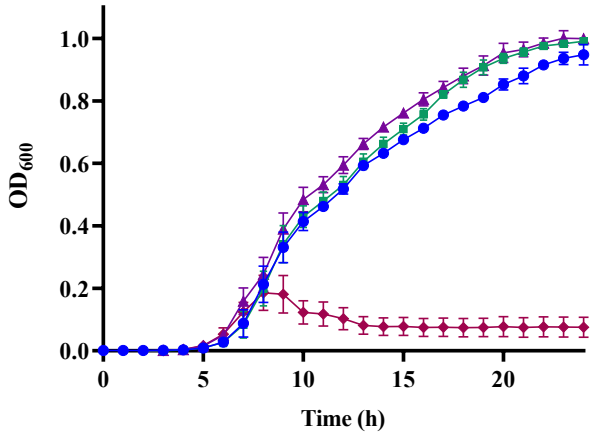
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	11.6[a]	12.12[a]	12.37[a]	1.95[b]
(SEM)	(0.1)	(0.09)	(0.1)	(0.1)

F) 8 µg/ml ceftiofur added at 8 hours



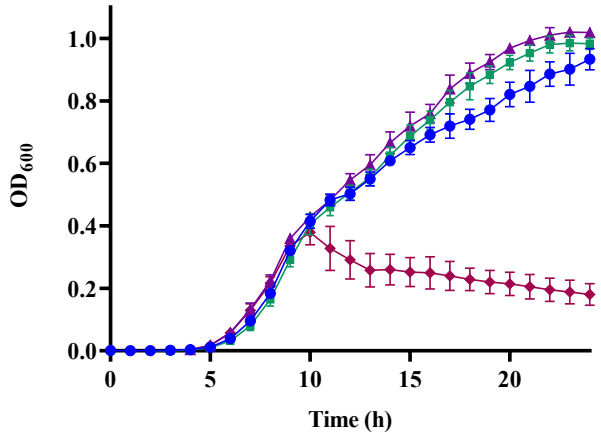
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	11.2[a]	11.21[a]	12.01[a]	6.69[b]
(SEM)	(0.1)	(0.17)	(0.1)	(0.28)

G) 16 µg/ml ceftiofur added at 5 hours



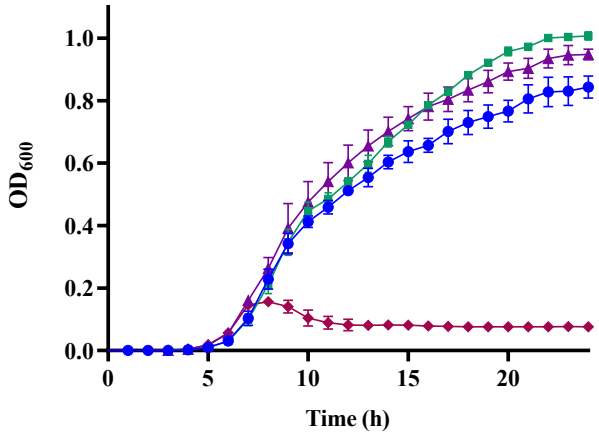
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	11.1[a]	11.8[a]	12.46[a]	1.78[b]
(SEM)	(0.14)	(0.15)	(0.15)	(0.19)

H) 16 µg/ml ceftiofur added at 8 hours



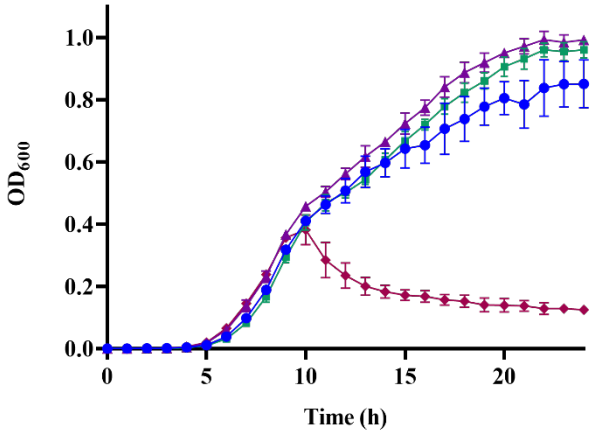
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC	10.71[a]	11.42[a]	12.15[a]	4.36[b]
(SEM)	(0.16)	(0.15)	(0.13)	(0.22)

I) 32 µg/ml ceftiofur added at 5 hours



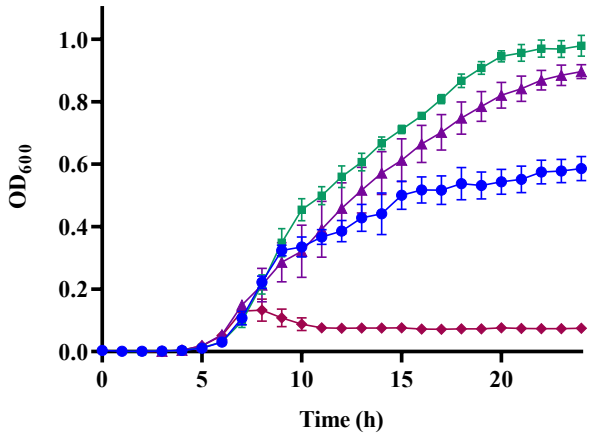
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	10.38[a] (0.17)	12.02[a] (0.1)	12.04[a] (0.23)	1.69[b] (0.1)

J) 32 µg/ml ceftiofur added at 8 hours



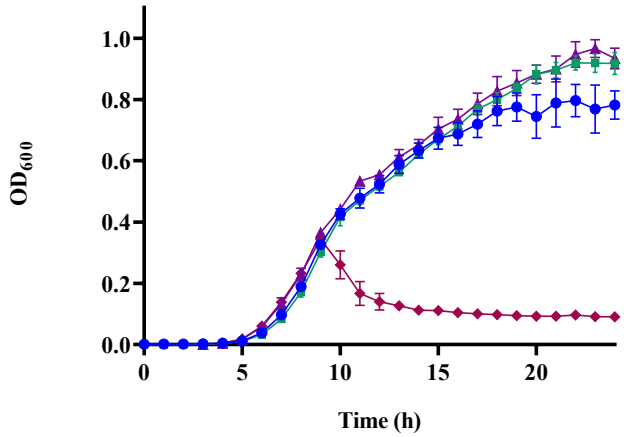
	CMY-2	CTX-M-15	KPC-3	Control
	10.43[a] (0.23)	11.19[a] (0.12)	12.17[a] (0.12)	3.5[b] (0.13)

K) 64 µg/ml ceftiofur added at 5 hours



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	7.81[a] (0.2)	11.87[b] (0.14)	10.36[c] (0.30)	1.56[d] (0.07)

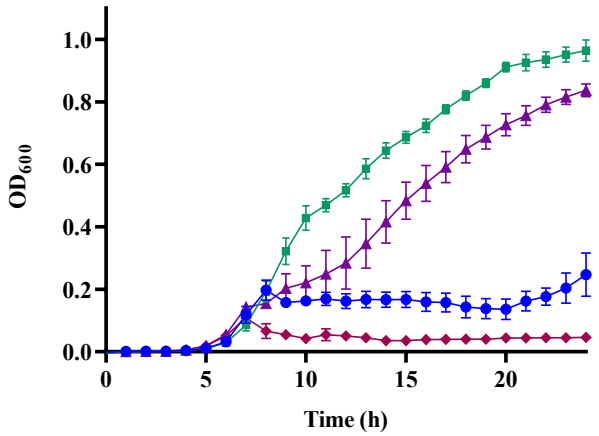
L) 64 µg/ml ceftiofur added at 8 hours



	CMY-2	CTX-M-15	KPC-3	Control
	10.43[a] (0.23)	11.05[a] (0.11)	11.67[a] (0.16)	2.53[b] (0.1)

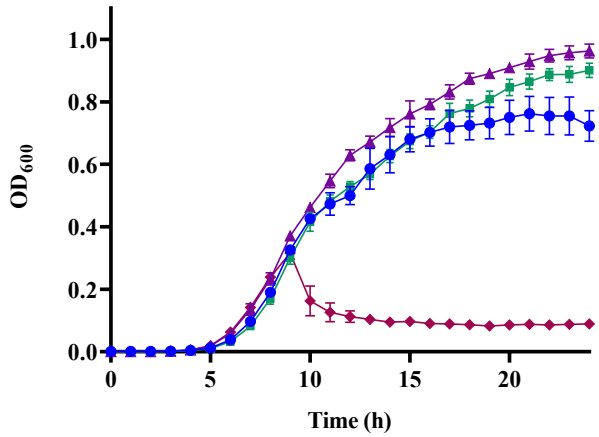
bla$_{CMY-2}$ *bla*$_{CTX-M-15}$ *bla*$_{KPC-3}$ pMMB207Δ*bla*$_{Tem-1}$ (control)

K) 128 µg/ml ceftiofur added at 5 hours



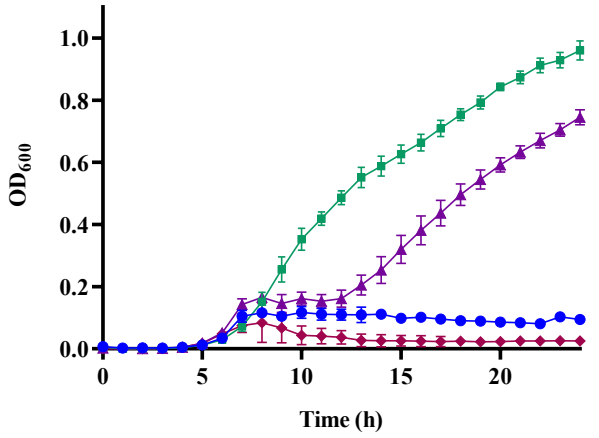
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	2.92[a] (0.16)	11.36[b] (0.13)	8.56[c] (0.26)	0.93[d] (0.1)

L) 128 µg/ml ceftiofur added at 8 hours



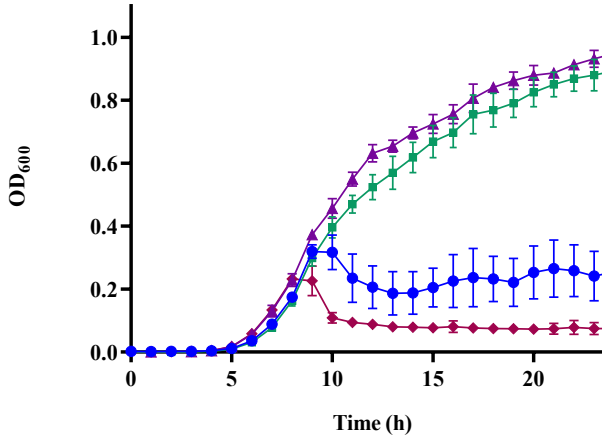
	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	10.23[a] (0.23)	10.88[a] (0.12)	12.22[b] (0.11)	2.22[c] (0.1)

K) 256 µg/ml ceftiofur added at 5 hours



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	1.82[a] (0.1)	10.5[b] (0.14)	6.61[c] (0.16)	0.7[d] (0.13)

L) 256 µg/ml ceftiofur added at 8 hours



	CMY-2	CTX-M-15	KPC-3	Control
x̄ AUC (SEM)	4.04[a] (0.35)	10.71[b] (0.22)	11.87[c] (0.13)	1.85[d] (0.1)