Supplementary Materials:

Table S1. MIC analysis of citric acid against five representative strains.

Pathogens	MIC (μg/mL)
E. coli ATCC 25922	>512
E. coli B2	>512
E. coli O157:H7	>512
S. aureus ATCC 29213	>512
MRSA T144	>512

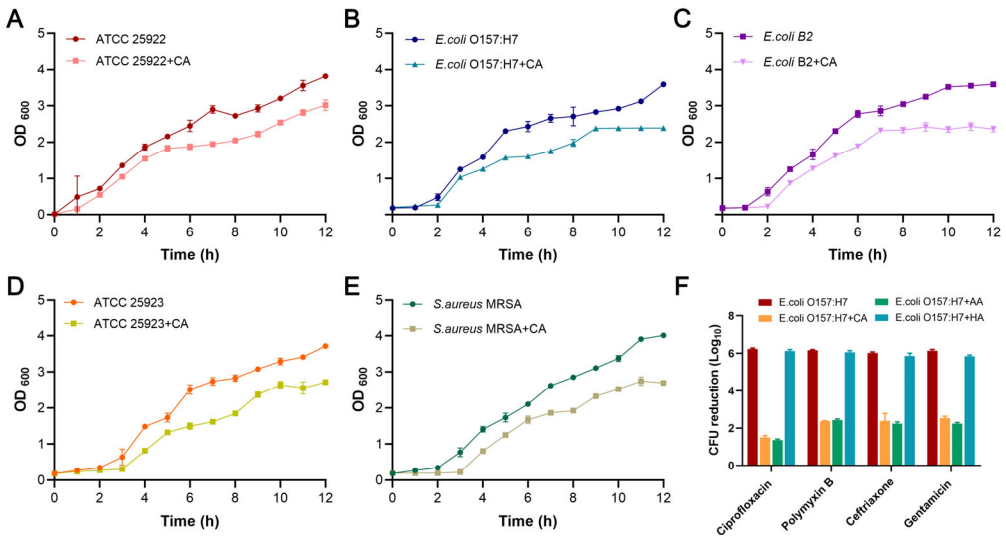

Figure S1 Growth curves of five representative strains grown in the presence of citric acid (final concentration 2%) for 12 hours. The optical density (OD_{600}) of citric acid and (A) *E.coli* ATCC 25922, (B) *E.coli* O157:H7, (C) *E.coli* B2, (D) *S.aureus* ATCC 25923, (E) MRSA T144 were monitored within 12 hours.（F）：Effect of adding different acidifiers on bactericidal activity of bactericidal antibiotics. CA: Citric acid, AA: Acetic acid, HA: Hydrochloric acid. After adding three acidifiers, the pH of the culture medium is about 2.2. Three biological replicates were used for the experiment.

Table S2 Co-culture of five representative strains with citric acid had no effect on MIC value of drugs

μg/mL

Antibiotic	Targets	*E. coli* ATCC 25922		*E. coli* O157:H7		*E. coli* B2		*S. aureus* ATCC 29213		MRSA T144	
		MIC [a]	MIC [b]	MIC [a]	MIC [b]	MIC [a]	MIC [b]	MIC [a]	MIC [b]	MIC [a]	MIC [b]
CIP	DNA synthesis	0.03	0.03	<0.03	<0.03	32	32	0.5	0.5	8	16
CRO	Cell wall	0.03	0.03	<0.03	<0.03	256	256	4	4	>512	>512
PB	Cell membrane	0.5	1	0.5	0.5	2	2	-	-	-	-
GEN	Protein synthesis	0.5	0.5	0.5	0.5	64	64	0.5	0.5	128	128
KAN	Protein synthesis	2	2	2	2	512	512	2	2	512	512
TIG	Protein synthesis	0.125	0.125	0.03	0.03	0.5	0.5	0.125	0.125	0.125	0.125
VAN	Cell wall	-	-	-	-	-	-	0.5	1	0.5	0.5

[a] Initial MIC of representative strains against antibiotics;

[b] MIC of representative strains after co-culturing with citric acid (final concentration 2%) for 6 h.

Abbreviations: CIP, Ciprofloxacin; CRO, Cefatriaxone; PB, Polymyxin B; GEN, Gentamicin; KAN, Kanamycin; TIG, Tigecycline; VAN, Vancomycin.

Table S3 MIC analysis of five antibiotics before and after citric acid induced antibiotic tolerance in *E.coli* B2.

Antibiotic	MIC (μg/mL)[a]	MIC (μg/mL)[b]
Cefatriaxone	256	256
Ciprofloxacin	32	32
Polymyxin B	2	2
Gentamicin	64	64
Kanamycin	512	512

[a] Initial MIC of *E.coli* B2 against antibiotics;

[b] MIC of CA-*E. coli* B2 induced by citric acid.

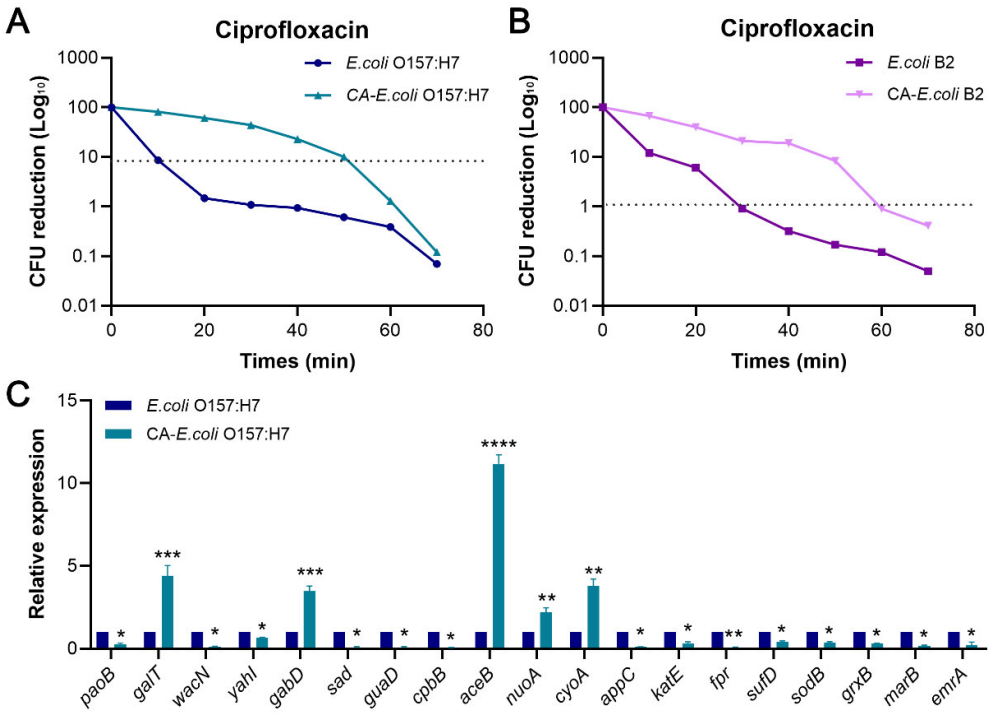

Figure S2: Growth status and mRNA expression of antibiotic tolerance strains induced by citric acid. (A and B): Determination of the growth rate of E. coli and CA-E. coli strains after stable inheritance of citric acid-induced antibiotic tolerance. Observe the absorbance value at OD_{600} nm of different strains within 12 hours. Three biological replicates are used. (C): The transcriptome sequencing results of the strains before and after citric acid induction were verified by RT-qPCR. All data are presented as mean ± SD and the significances determined by unpaired t test. All data from biological experiments performed in triplicate are presented as means ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

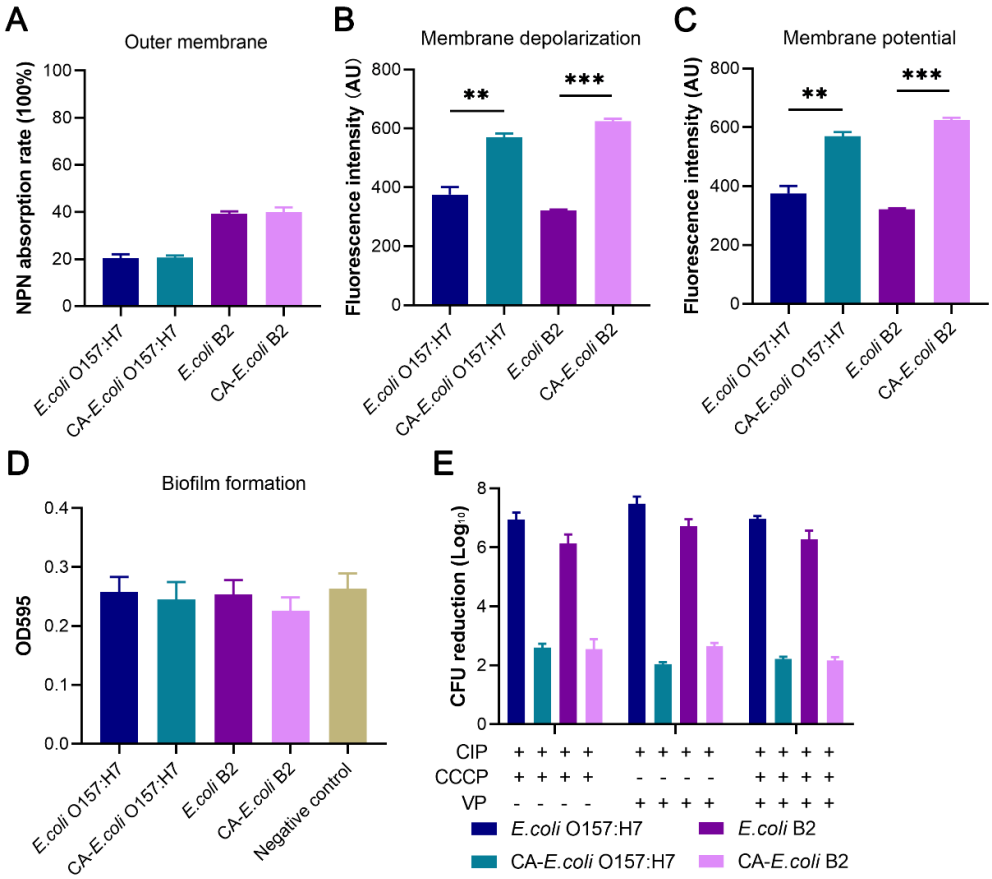

Figure S3: **Effects of citric acid-induced antibiotic tolerance on outer membrane integrity, membrane permeability, membrane potential changes and biofilm formation of *E. coli* strain.** (A and D): The outer membrane integrity and biofilm formation of the *CA-E.coli* strain did not change compared to the *E.coli* strain. (B and C): However, in the CA-*E. coli* strain, membrane depolarization and membrane potential dissipation occurred. (E): CCCP (efflux pump inhibitor, 100 μM) and VP (calcium channel inhibitor, 100 μM) alone or in combination did not affect the bactericidal activity of ciprofloxacin against CA-*E. coli*. All data from biological experiments performed in triplicate are presented as means ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$.

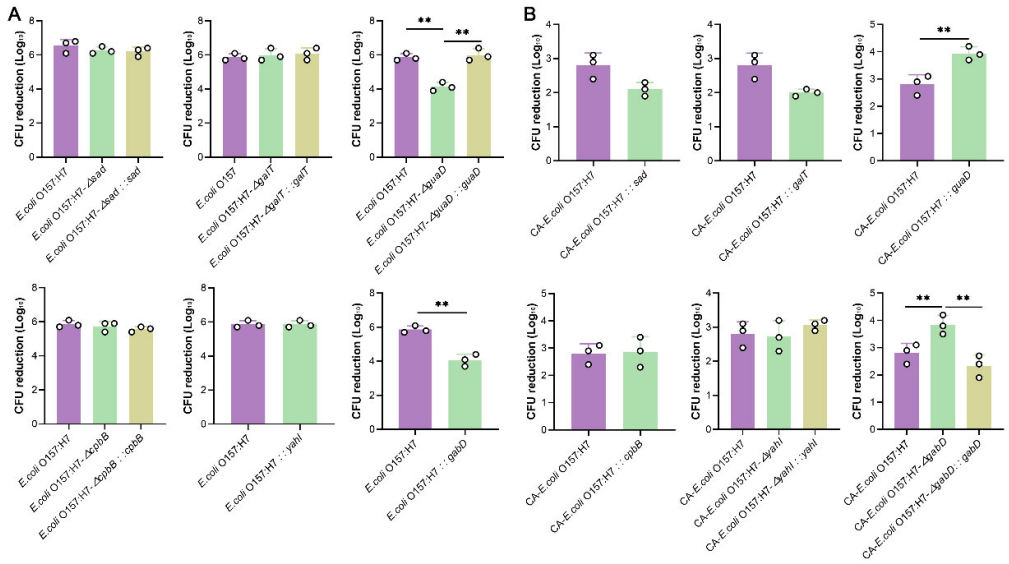

Figure S4: **Effects of *guaD* gene and *gabD* gene on antibiotic tolerance in *E.coli*.** A: Deletion and deletion-complemented of *guaD* gene and overexpression of *gabD* gene were constructed in *E.coli* O157:H7 strain. It was found that deletion of the *guaD* gene and overexpression of the *gabD* gene could improve antibiotic tolerance to some extent. B: Overexpression of the *guaD* gene , deletion and deletion-complemented of the *gabD* gene were constructed in CA-*E.coli* O157:H7 strain. It was also found that overexpression of the *guaD* gene and deletion of the *gabD* gene could restore the tolerance of the strain to ciprofloxacin to a certain extent. All data from biological experiments performed in triplicate are presented as means ± SD. $^{**}P < 0.01$.

Table S4 Strains used in this study.

Strains	Source/Reference
Gram-negative bacteria	
E. coli ATCC 25922	ATCC
E. coli O157:H7	In this study
E. coli B2 (*mcr-1* + bla_{NDM-5})	In this study
E. coli O157:H7 Δ*galT*	In this study
E. coli O157:H7 Δ*galT :: galT*	In this study
E. coli O157:H7 Δ*wcaN*	In this study
E. coli O157:H7 Δ*wcaN :: wcaN*	In this study
E. coli O157:H7 Δ*sad*	In this study
E. coli O157:H7 Δ*sad :: sad*	In this study
E. coli O157:H7 Δ*guaD*	In this study
E. coli O157:H7 Δ*guaD :: guaD*	In this study
E. coli O157:H7 Δ*cpbB*	In this study
E. coli O157:H7 Δ*cpbB :: cpbB*	In this study
E. coli O157:H7 *:: yahI*	In this study
E. coli O157:H7 *:: gabD*	In this study
Gram-positive bacteria	
S. aureus ATCC 29213	ATCC
MRSA T144	In this study

ATCC, American Type Culture Collection.

Table S5 RT-qPCR primers used in this study.

Genes	Sequence (5'→3')	Product (bp)
paoB	AAGGTGTTTTCAGCGGTGGGATG CTGGGCGGAGTAGCACATAAGC	122
yahI	CGGCATCGGCTATCTGATCCAAC TCCACTTCCACCTGAGTCACCAC	96
wcaN	GCAGCCACAATCTCGTCAACAATG GCAGTTATTCCTGTAGCGGGTCTC	125
sad	ATTCAGCCAGCCATATACGCCTTG TTCCCATCATTCTTGCAGGTAACGG	128
galT	AGCTGCCAGTGTTGATTCTCTTCG GACAACCTCTTCCAGTGCTCCTTC	86
cpdB	AGCACGAAACCACAGTCACTCATC CCTGACGGTTGATCTTCGCT	102
guaD	TGCCTGGGTGAAATCGCTTTATCC AGATGGACGCAGTGAGCAAAGAC	105
fixA	GTATGGTCGGCGGCGGATATTG GCAAATGCGGCGATCTGTTCTTC	134
fixX	CAAGAAGCAGGATGACGGCAGTG CGTAACGGAACTCCACACCAAAGG	140
folA	TGACGCATATCGACGCAGAAGTG CCGCCGCTCCAGAATCTCAAAG	143
polB	AGTTCACCCGCCATCAGTTTGTC GCCCAGCAGTTTCAGCAGGAG	101
copA	TACGCTCCGCCAGGTTTACCC ATGACGATGACAGCCAGCAGTTG	126
degP	CAACGGCGGCTGAGACTTCTTC GCTGACCACTGAAGGCATCACC	92
rclA	TCAATGCCCATGTGGAGCGAATC CTGGATGTAACGAAGCGGTAGCC	132
csgA	CGGTGGTGGCGGTAATAATAGCG GTCAAGTCAGAGTTACGGGCATCAG	111
ydcL	CACCGAAATGAAAGAAGCGATAGCG GCCCCACGGAAAATCAGACTACG	81
aceF	AGAACGAAGAAGCGGCGAAACG AGCGAACTATTGAAGCGAGGCATC	112
emrA	CGTGCGTCTTCCTCGGTAATGTAG CACCTCTTCTATCGGCGTTGTCAC	117
16S rRNA	AACTGGAGGAAGGTGGGGATGAC CGGACTACGACGCACTTTATGAGG	135