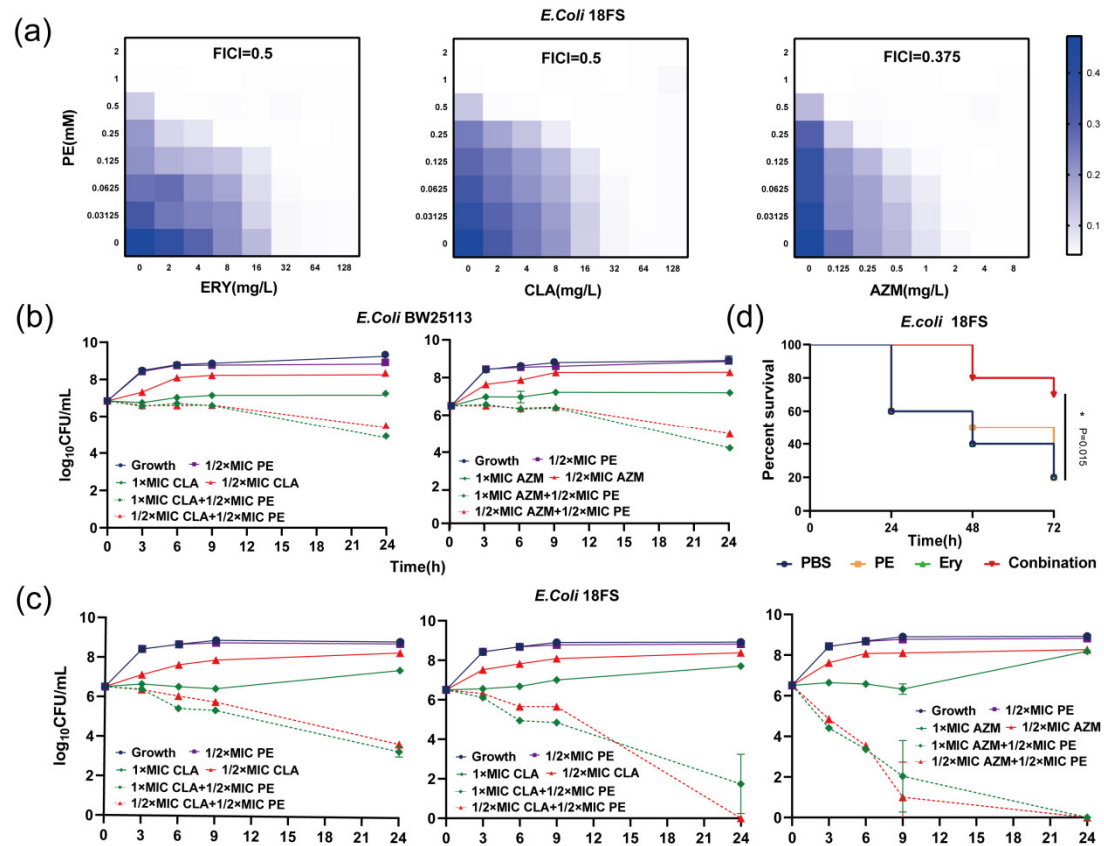


## Supplementary File



**Figure S1** (a) In vitro testing of phentolamine combined with 3 macrolide antibiotics (erythromycin, clarithromycin and azithromycin) indicated a synergistic action against *E. coli* strains with FICI values 0.5, 0.5 and 0.375. (b) The time–kill curves representing log<sub>10</sub> changes in bacterial burden of *E. coli* BW25113 over 24 h, following exposure to phentolamine (1/2×MIC) in the presence of increasing clarithromycin and azithromycin concentrations (1/4 –1/2×MIC) are shown. (c) The time–kill curves representing log<sub>10</sub> changes in bacterial burden of *E. coli* 18FS over 24 h, following exposure to phentolamine (1/2×MIC) in the presence of increasing erythromycin, clarithromycin and azithromycin concentrations (1/4 -1/2×MIC). (d) Phentolamine can improve the antibacterial activity of erythromycin against *E. coli* 18FS in vitro.

**Table S1.** Bacterial strains, plasmids, and primers used in this study.

Strains, Plamids and Primers		Relevant Features and Use
pCasKP-apr		Bacterial expression of Cas9 nuclease and $\lambda$ -Red system
PKD4		Bacterial expression; Gene knockout system
tolCF	5-TACAGTTTGATCGCGCTAAATACTGCTTCACCACAAGGAATGCAAGCAGCATTACACGTCTTGAG-3	
tolCR	5-TACGTTGCCTTACG TTCAGACGGGGCCGAAGCCCCGTCGTCGTCAGCTGACATGGGAATTAGCCA-3	

**Table S2.** In vitro antimicrobial susceptibility profiles for test strain. MIC values of 11 antibiotics against *E.coli* 18FS. Gentamicin (GEN), Amikacin (AMK), Meropenem (MEM), Ceftaxime (CTX), Ceftazidime (CAZ), Cefoxitin (FOX), Ciprofloxacin (CIP), Florfenicol (FFC), Tetracycline (TET), Sulfamethoxazole/Trimethoprim (SMZ/TMP), Colistin (CS).

Test Strain	Species	MIC (mg/L)										CS
		GEN	AMK	MEM	CTX	CAZ	FOX	CIP	FFC	TET	SMZ/ TMP	
18FS	<i>E. coli</i>	128	8	256	>256	>256	>256	256	256	256	>304/16	4