Synergistic activity of colistin combined with PFK-158 against colistin-resistant and colistin-susceptible Gram-negative bacteria

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Research

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Abstract

Background: The emergence of colistin resistance among Gram-negative bacteria poses a serious public health threat and warrants immediate action. Therefore, it is necessary to enhance the antibacterial activity of colistin through the combination with other drugs. In this study, we demonstrate the synergistic activity of colistin combined with PFK-158 against colistin-susceptible but more importantly against colistin-resistant Gram-negative bacteria, including non-fermenting bacteria (*P. aeruginosa*, *A. baumannii*) and *Enterobacteriaceae* (*E. coli* and *K. pneumoniae*).

Methods: 18 colistin-resistant and 12 colistin-susceptible Gram-negative bacteria were collected as the experimental strains, and the minimum inhibitory concentrations (MICs) of colistin and PFK-158 against all strains were determined by the broth microdilution method. The MICs of routine antimicrobial agents including aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), imipenem (IMP), ciprofloxacin (CIP), levofloxacin (LVX), gentamicin (GEN), tobramycin (TOB) for all 30 experimental strains were determined by bioMerieux VITEK-2 (BioMérieux, Marcy-l'Étoile, France). The synergistic activity of colistin combined with PFK-158 *in vitro* was assessed using the checkerboard assay and the time-kill assays.

Results: The results of the checkerboard assay showed that when colistin was used in combination with PFK-158, synergistic activity was observed against the 18 colistin-resistant and the 8 colistin-susceptible Gram-negative bacteria, and the remaining 4 colistin-susceptible strains showed additive activity. No irrelevant activity and antagonistic activity was observed for all strains. The results of the time-killing assays presented that the killing activity against the colistin-resistant Gram-negative bacterium were evident for the combination of colistin and PFK-158, compared with the groups adding colistin or PFK-158 alone.

Conclusions: In conclusion, our results strongly exhibited that the combination of colistin and PFK-158 displayed the significant synergistic activity against all tested colistin-resistant and most colistin-susceptible Gram-negative strains. PFK-158 was found to potentiate the antibacterial activity of colistin against a wide panel of colistin-resistant and colistin-susceptible Gram-negative strains no matter what species (including non-fermenting bacteria and *Enterobacteriaceae*). It may be a potential new choice for the treatment of infections caused by the clinical Gram-negative strains.

Background

Because of the rapid spread of antimicrobials resistance and the slow development of novel antimicrobials, Gram-negative bacteria infections are becoming extremely challenging for clinicians and a real threat to international public health [1, 2]. Rapid spread and dissemination of resistance-encoding genes among pathogens further exacerbate the problem, resulting in an ever increasing rate of antimicrobial-resistant bacterial infections [3, 4]. The traditional drug, colistin is one of the defensive drugs commonly used clinically to treat infections caused by multi-drug-resistant and carbapenem-resistant Gram-negative bacteria [5]. Unfortunately, the increased and inappropriate use of colistin has led

inexorably to the worldwide emergence of colistin-resistant Gram-negative bacteria in the last few years [6, 7, 8]. The emergence of colistin resistance among Gram-negative bacteria poses a serious public health threat and warrants immediate action [9, 10]. Therefore, it is necessary to enhance the antibacterial activity of colistin through the combination with other drugs.

PFK-158 is a potent and selective 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3 (PFKFB3) inhibitor, which shows extensive anti-tumor activity by reducing the uptake of glucose in cancer cells, the production of ATP, the release of lactic acid and inducing apoptosis and autophagy [11, 12]. A phase I clinical trial of PFK-158 was successfully completed in July 2016.

At present, there was only one study having investigated the synergistic activity of colistin combined with PFK-158 against colistin-resistant *Enterobacteriaceae* [13]. However, there was no study exploring the synergistic activity of combination of this two drugs against colistin-resistant *P. aeruginosa*, *A. baumannii* and colistin-sensitive Gram-negative bacteria. Therefore, the main aim of this study was to determine the synergistic activity of colistin in combination with PFK-158 against colistin-resistant and colistin-susceptible Gram-negative bacteria including *P. aeruginosa*, *A. baumannii* and *Enterobacteriaceae*, in order to provide new possible future therapeutic strategies to combat resistance to this antibiotic of last resort.

Methods

Bacterial strains

A total of 30 non-duplicated Gram-negative clinical isolates were recovered from the First Affiliated Hospital of Wenzhou Medical University in China, including colistin-resistant *P. aeruginosa* (n = 9), colistin-susceptible *P. aeruginosa* (n = 3), colistin resistant *A. baumannii* (n = 3), colistin-susceptible *A. baumannii* (n = 3), colistin-resistant *E. coli* (n = 3), colistin-susceptible *E. coli* (n = 3), colistin-resistant *K. pneumoniae* (n = 3) and colistin-susceptible *K. pneumoniae* (n = 3). These isolates were all identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS; bioMérieux, Lyons, France). All strains were stored in Luria Bertani (LB) broth medium at -80 °C containing 30% glycerol for later use. *P. aeruginosa* ATCC 27853 and ATCC 25922 were served as the quality controls, which were purchased from the national center of clinical laboratory (NCCL).

Antimicrobial Susceptibility Assay

PFK-158 is dissolved in dimethyl sulfoxide (DMSO). In order to avoid the influence of the antibacterial activity of DMSO, the final concentration of DMSO should not exceed 5% (v/v). The MICs of colistin and PFK-158 were determined by the broth microdilution in cation-adjusted Mueller-Hinton Broth (CAMHB). The CAMHB was prepared by adding an appropriate amount of Mg^{2+} and Ca^{2+} into Mueller-Hinton Broth, with final concentrations of 10-12.5 mg/L and 20–25 mg/L, respectively. The final bacteria concentration of each well was approximately at 5×10^5 colony forming units (CFU)/mL. Serial two-fold dilutions ranging from 128 to 0.0625 μg/ml for colistin, and 1024 to 0.5 μg/ml for PFK-158 were prepared in

CAMHB 96-well microtiter plates. The results were observed after incubation at 37 ℃ for 16–18 h. The MICs of colistin determination for all strains were interpreted by the recommendation of the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (http://www.eucast.org/) and CLSI (susceptible, ≤ 2 μg/ml; resistant, > 2 μg/ml). Besides, the MICs of routine antimicrobial agents including aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), imipenem (IMP), ciprofloxacin (CIP), levofloxacin (LVX), gentamicin (GEN), tobramycin (TOB) for all 30 experimental strains were determined by bioMerieux VITEK-2 (BioMérieux, Marcy-l'Étoile, France). Multidrug resistant (MDR) strains were defined as non-susceptible to three or more different antimicrobial categories [14]. All experiments were performed in triplicate.

Checkerboard Assay

The *in-vitro* synergistic activity of the combination against the colistin-resistant and colistin-susceptible Gram-negative bacteria was examined by using the checkerboard assay on a 96-well plate as previously described. Referring to the MICs of the above two drugs, colistin is 2-fold serially diluted along the x-axis, while PFK-158 is 2-fold serially diluted along the y-axis to form a matrix, where each well is composed of two drugs with different concentrations. The single bacterial colony grown overnight was diluted to 0.5 McDonnell turbidity in sterile saline, and subsequently followed by 1:100 further dilution in CAMHB. And then 100 μl of bacterial suspension was added to the corresponding well to achieve a final concentration of approximately 5×10^5 CFU /ml. Wells containing CAMHB with or without bacterial cells were used as positive or negative controls, respectively. The 96-well plates were then incubated at 37 °C for 16–18 h and record the MICs of the single drug and the MICs of the combination of colistin with PFK-158.

The fractional inhibitory concentration index (FICI) was used to evaluate the potential synergistic activity of the two drugs in combination. The FIC index was calculated with the formula: FICI = (MIC of drug A in the combination/MIC of drug A alone) + (MIC of drug B in the combination/MIC of drug B alone). FICI ≤ 0.5, 0.5 < FICI ≤ 1.0, 1.0 < FICI ≤ 2.0, FICI > 2.0 was expected as synergistic activity, additive activity, irrelevant activity, and antagonistic activity, respectively. All experiments were performed in triplicate.

Time-kill Assay

The time-kill assay was conducted according to a published protocol with several modifications [15]. In brief, we randomly selected 9 strains in the colistin-resistant Gram-negative bacteria as experimental strains, including colistin-resistant *P. aeruginosa* (n = 6), *A. baumannii* (n = 1), *E. coli* (n = 1) and *K. pneumoniae* (n = 1). The synergistic activity of these two drugs was achieved with combinations of colistin and PFK-158 used at the lowest concentration when it showed synergistic activity for the 9 experimental strains. At the same time, we set up the control group adding colistin and PFK-158 alone. Viable cells were counted by spreading 100 μL of samples after appropriate dilutions with saline and plating on antibiotic-free Mueller-Hinton agar plates at 0, 2, 4, 6, 12 and 24 h after antibiotic addition. Bacterial colonies were counted from the plates after incubation for 16–18 h at 37 °C. The assay was performed in triplicate for these strains, then we calculate the average value of viable CFU and plot it on a

semi-logarithmic graph. Antimicrobial carryover was controlled by the inhibition of colonial growth at the site of the initial streak according to NCCLS guidelines [15]. Bactericidal activity was defined as a ≥ 3 log10 decrease in CFU/mL by 24 h. Synergistic activity was defined as a ≥ 2 log10 decrease in CFU/mL at 24 h by the two drugs combination when compared with the single drug [15].

Results

Antimicrobial agents and susceptibility test

As shown in Table 1, twelve of 18 colistin-resistant strains and five of 12 colistin-susceptible strains exhibited multidrug-resistance.

Table 1
Representative MIC values against colistin-resistant and colistin-susceptible Gram-negative bacteria.

Species	Strains	MIC (μg/mL)							
		ATM	CAZ	FEP	IPM	CIP	LVX	GEN	TOB
P. aeruginosa	TL1671	2	2	4	4	1	0.5	4	1
	TL1722	16	2	2	16	2	2	2	0.25
	TL1736	1	0.5	0.5	16	0.5	0.5	32	8
	TL1744	32	32	8	16	8	8	64	64
	TL2314	16	4	16	4	0.5	0.5	8	1
	TL2917	32	8	8	32	1	1	8	1
	TL2967	64	8	16	16	8	16	4	1
	TL3008	4	4	4	16	1	8	32	4
	TL3086	4	2	4	16	0.5	1	32	4
	TL2231	16	4	8	16	2	2	4	1
	TL2595	8	4	2	1	0.25	0.5	1	1
	TL2911	16	4	2	1	0.25	2	1	1
A. baumannii	BM2370	16	4	2	1	0.25	0.25	1	1
	BM2431	64	64	64	16	4	8	1	1
	BM1539	16	8	64	16	4	2	1	1
	BM4909	8	8	8	1	0.5	0.25	1	1
	BM5036	16	4	4	1	0.25	0.25	1	1
	BM5073	1	8	4	1	0.5	0.25	1	1
E. coli	DC90	8	16	8	1	4	8	16	8
	DC3411	16	16	8	1	4	8	1	1
	DC3539	4	16	2	1	4	8	16	16
	DC9032	64	64	64	1	4	8	16	8
	DC9033	64	64	64	1	4	8	16	16
	DC9034	2	2	1	1	4	8	16	8

ATM, Aztreonam; CAZ, Ceftazidime; FEP, Cefepime; IMP, Imipenem; CIP, Ciprofloxacin; LVX, Levofloxacin; GEN, Gentamicin; TOB, Tobramycin

K. pneumoniae	FK1342	64	64	64	16	4	16	16	16
	FK26	64	64	64	32	4	8	16	16
	FK6556	64	64	64	16	4	8	16	16
	FK5135	1	1	1	1	0.25	0.25	1	1
	FK5156	64	64	64	16	4	8	16	16
	FK5157	64	64	64	16	4	8	16	16

ATM, Aztreonam; CAZ, Ceftazidime; FEP, Cefepime; IMP, Imipenem; CIP, Ciprofloxacin; LVX, Levofloxacin; GEN, Gentamicin; TOB, Tobramycin

Synergistic activity testing by the checkerboard assay

It was confirmed by the checkerboard assay that there were 18 colistin-resistant(> 2 μg/ml) and 12 colistin-susceptible (≤ 2 μg/ml) Gram-negative bacteria. In addition, the MICs of PFK-158 against all 30 tested strains were 512 μg/ml. FICI of < 0.5 was found for all 18 colistin-resistant Gram-negative bacteria (Table 2), which include *P. aeruginosa* (n = 9), *A. baumannii* (n = 3), *E. coli* (n = 3) and *K. pneumoniae* (n = 3), indicating significant synergistic activity of colistin combined with PFK-158 against colistin-resistant Gram-negative bacteria. And we observed that the combination of colistin and PFK-158 could reduce colistin MICs of the colistin-resistant strains by 4 to 16 folds. Simultaneously ,as for the 12 colistin-susceptible Gram-negative bacteria including *P. aeruginosa* (n = 3), *A. baumannii* (n = 3), *E. coli* (n = 3) and *K. pneumoniae* (n = 3), there was no significant difference of the synergistic activity, compared with the colistin-resistant Gram-negative bacteria. The checkerboard assay result presented that there were 9 strains showing synergistic activity (9/12), and the remaining 3 strains displayed additive activity (3/12) (Table 3). Besides, 2–16 folds reducation of colistin MICs was observed in colistin combined with PFK-158 against the colistin-susceptible strains. In brief, there was no irrelevant activity and antagonistic activity being observed above all 30 experimental strains.

Table 2
Summary of MIC and FICI of colistin combined with PFK-158 against 18 colistin-resistant GNB.

Colistin-resistant GNB	Strains	Antimicrobial susceptibility (MIC,μg/mL)		Antimicrobial combination (MIC, μg/mL)			
		CST	PFK-158	CST + PFK-158	FICI	Potentiation	Interpretation
P. aeruginosa	TL1671	8	512	1 + 16	0.16	8-fold	Synergy
	TL1722	8	512	2 + 16	0.28	4-fold	Synergy
	TL1736	16	512	4 + 16	0.28	4-fold	Synergy
	TL1744	8	512	2 + 16	0.28	4-fold	Synergy
	TL2314	8	512	2 + 16	0.28	4-fold	Synergy
	TL2917	16	512	4 + 32	0.31	4-fold	Synergy
	TL2967	8	512	2 + 32	0.31	4-fold	Synergy
	TL3008	32	512	8 + 16	0.28	4-fold	Synergy
	TL3086	32	512	8 + 32	0.31	8-fold	Synergy
A. baumannii	BM2370	16	512	1 + 8	0.08	16-fold	Synergy
	BM2431	32	512	4 + 16	0.16	8-fold	Synergy
	BM1539	16	512	4 + 32	0.31	4-fold	Synergy
E. coli	DC90	8	512	2 + 16	0.28	4-fold	Synergy
	DC3411	8	512	1 + 32	0.19	8-fold	Synergy
	DC3539	32	512	4 + 16	0.16	8-fold	Synergy
K. pneumoniae	FK1342	> 128	512	8 + 32	0.13	16-fold	Synergy
	FK26	> 128	512	16 + 16	0.16	8-fold	Synergy
	FK6556	32	512	4 + 32	0.19	8-fold	Synergy

GNB, Gram-negative bacteria;MIC, minimum inhibitory concentration; CST, colistin; FICI, fractional inhibitory concentrations index.

Table 3
Summary of MIC and FICI of colistin combined with PFK-158 against 12 colistin- susceptible GNB and 2 quality control strains.

Colistin-susceptible GNB	Strains	Antimicrobial susceptibility (MIC, μg/mL)		Antimicrobial combination (MIC, μg/mL)			
		CST	PFK-158	CST + PFK158	FICI	Potentiation	Interpretation
P. aeruginosa	TL2231	1	512	0.5 + 16	0.53	2-fold	Additivity
	TL2595	2	512	1 + 32	0.53	2-fold	Additivity
	TL2911	2	512	0.5 + 16	0.28	4-fold	Synergy
ATCC 27853		1	512	0.5 + 16	0.53	2-fold	Additivity
A. baumannii	BM4909	2	512	0.125 + 8	0.08	16-fold	Synergy
	BM5036	1	512	0.25 + 16	0.28	4-fold	Synergy
	BM5073	1	512	0.125 + 32	0.19	8-fold	Synergy
E. coli	DC9032	1	512	0.125 + 16	0.16	8-fold	Synergy
	DC9033	1	512	0.25 + 16	0.28	4-fold	Synergy
	DC9034	0.5	512	0.0625 + 8	0.14	8-fold	Synergy
ATCC 25922		0.5	512	0.25 + 16	0.53	2-fold	Additivity
K. pneumoniae	FK5135	0.5	512	0.125 + 32	0.31	4-fold	Synergy
	FK5156	0.5	512	0.125 + 16	0.28	4-fold	Synergy
	FK5157	0.5	512	0.25 + 16	0.53	2-fold	Additivity

GNB, Gram-negative bacteria;MIC, minimum inhibitory concentration; CST, colistin; FICI, fractional inhibitory concentrations index.

Time–kill assay and strains activity

Time-kill curves against 9 colistin-resistant strains TL1671, TL1736, TL1744, TL2314, TL3008, TL3086, BM2431, DC3539 and FK1342 were revealed in Fig. 1. With colistin or PFK-158 alone, all colistin-resistant strains showed a regrowth after a reduction at 2 h. The combination of colistin and PFK-158 exhibited constant both synergistic and bactericidal activity for TL1736, TL2314, TL3008, TL3086, BM2431, DC3539 and FK1342 for 24 h. As for TL1671, synergistic activity was observed over 24 h for the combination of colistin and PFK-158, compared with colistin or PFK-158 alone. The combination showed a synergistic activity at 6 h against TL1744, but regrowth was observed at 24 h. In summary, this combination of colistin and PFK-158 enhanced their killing activity of drug exposure.

Discussion

The current epidemiological situation is especially worrying with MDR Gram-negative bacteria spreading worldwide and with a paucity of novel marketed antibiotics, thus colistin is gaining increasing interest from many clinicians worldwide [16]. However, the increasing numbers of discoveries of colistin-resistant pathogens broke the last barrier between superbacteria and humans and new therapy strategies are urgently required [17]. A large quantity of previous studies have reported the synergistic activity of colistin combined with other antibiotics against colistin-resistant or carbapenem-resistant strains [18, 19, 20]. Besides, a few studies based on colistin in combination with the nonantibiotics were also performed previously [21, 22, 23]. In this study, we evaluated the synergistic activity of colistin combined with PFK-158 against colistin-resistant and colistin-susceptible Gram-negative bacteria.

Firstly, as shown in Table 2, we noticed that for the 18 colistin-resistant Gram-negative bacteria, the addition of PFK-158 demonstrated the enhanced colistin susceptibility by reducing colistin MICs and fractional inhibitory concentration indices (FICIs) of the combination of colistin and PFK-158 were all below 0.5 in all 18 colistin-resistant strains, indicating that there was a synergistic activity of colistin combined with PFK-158. It should be emphasized that a synergistic activity was observed against all tested colistin-resistant *P. aeruginosa*, *A. baumannii*, *E.coli* and *K. pneumoniae*, suggesting that the combination may be a potential strategy to treat the infections caused by these four kinds of colistin-resistant Gram-negative bacteria. Recently, Xuefu You et al. have reported on the synergistic activity of colistin combined with PFK-158 against colistin-resistant *Enterobacteriaceae* [13], including *E.coli*, *K. pneumoniae* and *E. cloacae*, the result of which is consistent with the result in our study. Compared to the study of Xuefu You et al., the advantage of our study is that we have explored not only the synergistic activity of colistin in combination with PFK-158 against *Enterobacteriaceae* but also non-fermenting bacteria (*P. aeruginosa*, *A. baumannii*) and colistin-susceptible Gram-negative bacteria. We found that PFK-158 could potentiate colistin activity through a specific mechanism against all the colistin-resistant Gram-negative bacteria no matter what kinds of bacteria among the tested colistin-resistant *P. aeruginosa*, *A. baumannii*, *E. coli* and *K. pneumoniae*. In other words, the combination of colistin and PFK-158 showed a wide range of synergistic activity on colistin-resistant Gram-negative bacteria. This suggests that the mechanism of the synergistic activity of the two drugs combination is probably not related to the difference of the strains property.

However, data of the previous studies are available against only colistin- resistant strains, and little is known regarding the potential for the synergistic activity of colistin in combination with PFK-158 against colistin-susceptible Gram-negative bacteria. Therefore, to explore the reason why the combination of the two drugs could show synergistic activity and whether it is because PFK-158 changes the colistin resistance mechanism of the colistin-resistant Gram-negative bacteria, we further examined the synergistic activity of colistin combined with PFK-158 against colistin-susceptible Gram-negative bacteria as a comparison. The synergistic activity was also noted against most tested Gram-negative bacteria, and for the remaining susceptible Gram-negative bacteria, the combination displayed additive activity as shown in Table 3. When comparing the results between colistin-resistant and colistin-susceptible strains, no statistical significance was found ($p < 0.5$). This tells us that the mechanism of the synergy of the two drugs combination may be not related to the resistance mechanism of the strains.

So, what are the specific mechanism and reasons why the combination of colistin and PFK-158 can displayed the synergistic activity against colistin-resistant and colistin-susceptible Gram-negative bacteria? According to the research having been published, colistin is known to interact with lipopolysaccharide (LPS) and phospholipids present at the surface of the outer membrane, to disturb membrane permeability, and finally to bind to phospholipids present at the surface of the cytoplasmic membrane [24, 25]. The last interaction is thought to result in disruption of the osmotic equilibrium and leakage of the cell contents [26]. Besides, PFK-158 shows extensive anti-tumor activity by reducing the uptake of glucose in cancer cells, the production of ATP, the release of lactic acid and inducing apoptosis and autophagy [11, 12]. On the basis of the results as described above, the antibacterial mechanism of colistin and the anti-tumor mechanism of PFK-158, it reminded us that PFK-158 might confer colistin stronger ability to enter bacteria by influencing the metabolism of the strains so as to change the structure of lipid A moiety of LPS on the strains membrane, which is the main site of action of colistin. However, the mechanism of synergism observed between colistin and PFK-158 against colistin-resistant and colistin-susceptible Gram-negative bacteria remains unknown and will be the subjects of further investigations.

Finally, it is crucial to evaluate the clinical significance of these observations and to better understand the dose-response relationships of combination of colistin combined with PFK-158. As we all know, colistin has the nephrotoxicity and neurotoxicity [27], thus, if the combination therapy can lower the concentration and dosage of colistin, it will be extremely meaningful for the clinical treatment of colistin-resistant and colistin-susceptible Gram-negative bacteria infections, especially for the patients with cancer, who are a special group with the low immunity and are more prone to be infected.

Conclusions

In conclusion, our study indicated that *in vitro* substantial synergistic activity of colistin combined with PFK-158 against colistin-resistant and colistin-susceptible Gram-negative bacteria, including non-fermenting bacteria (*P. aeruginosa*, *A. baumannii*) and *Enterobacteriaceae* (*E. coli* and *K. pneumoniae*). Colistin in combination with PFK-158 may be a new alternative for the treatment of colistin-resistant and

colistin-susceptible Gram-negative bacterial infections. The mechanism of synergy of the two drugs combination may be not related to the resistance mechanism of the strains. In addition, PFK-158 might confer colistin stronger ability to enter bacteria by influencing the metabolism of the strains to change the structure of lipid A moiety of LPS on the strains membrane, which is the main site of action of colistin. And, it will be extremely meaningful for the clinical treatment of colistin-resistant and colistin-susceptible Gram-negative bacterial infections by reducing the concentration and dosage of colistin. So, further clinical study is recommended to assess the potential application of the combination of the two drugs in clinical practice.

Abbreviations

ATCC: American Type Cultures Collection; CLSI: Clinical and Laboratory Standards Institute; MICs: The minimum inhibitory concentrations; ATM: Aztreonam; CAZ: Ceftazidime; FEP: Cefepime; IMP: Imipenem; CIP: Ciprofloxacin; LVX: Levofloxacin ; GEN: Gentamicin; TOB: Tobramycin; PFKFB3: 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3; NCCL: The national center of clinical laboratory; DMSO: Dimethyl sulfoxide; CAMHB: Mueller-Hinton Broth; CFU: Colony forming units; MDR: Multidrug resistant; FICI: The fractional inhibitory concentration index; LPS: lipopolysaccharide; GNB: Gram-negative bacteria.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The need for ethics approval and consent is deemed unnecessary in this research according to the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Contributions

LQC conducted the experiments, analyzed the data and wrote the manuscript. KHY participated in experiments and writing. LJC and NH provided colistin-resistant and colistin-susceptible strains and participated in analysis of results. XKZ and YSL participated in analysis of results. TLZ and JMC helped design the study. HYJ and WLL designed the study and corrected the manuscript. All authors read and approved the manuscript.

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Figures

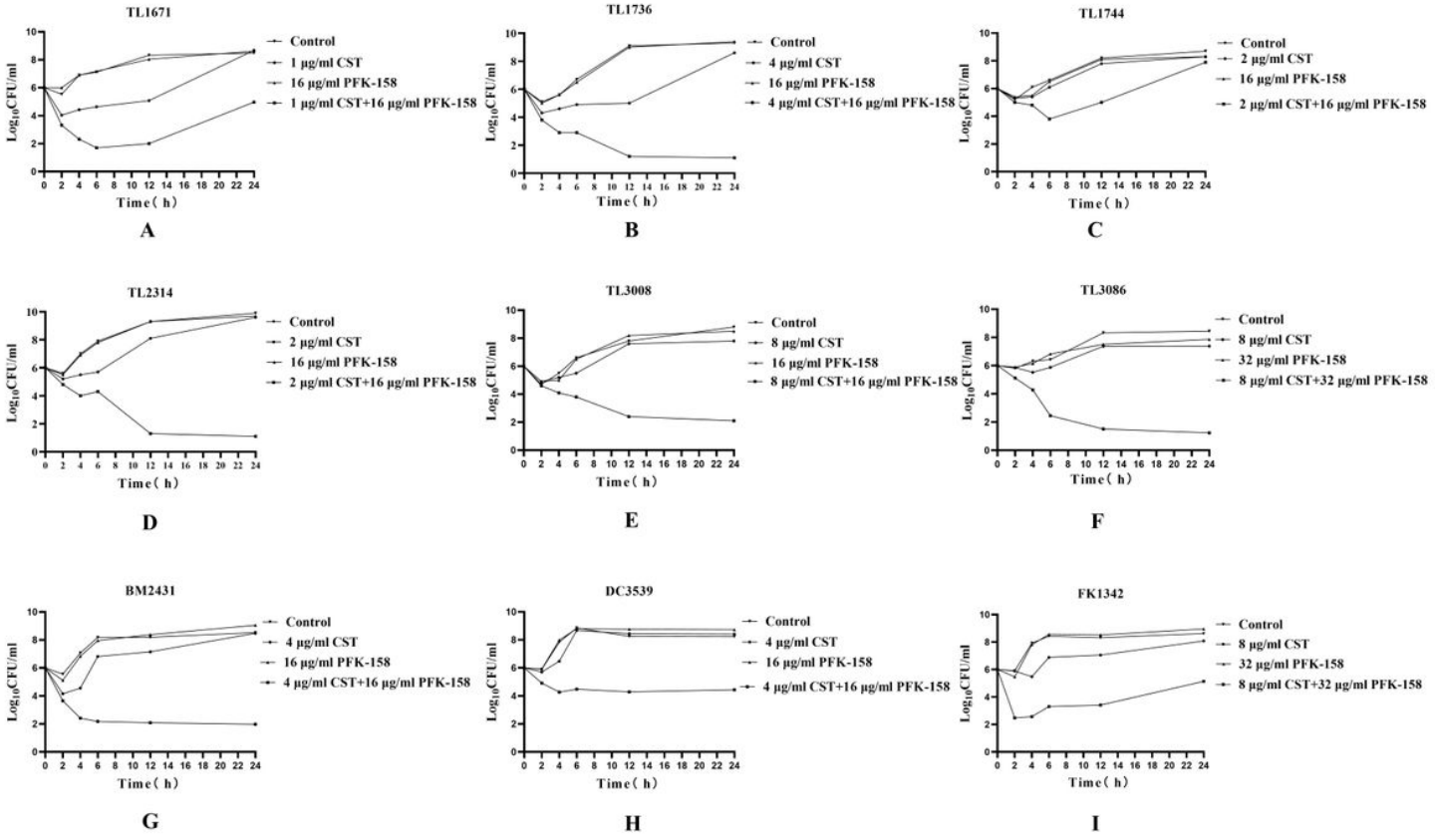

Figure 1

Time-killing curves. Time-killing curves of colistin and PFK-158 alone or in combination against TL1671(A), TL1736(B), TL1744(C), TL2314(D), TL3008(E), TL3086(F), BM2431(G), DC3539(H), FK1342(I).