Contents lists available at ScienceDirect

Arabian Journal of Chemistry

journal homepage: www.ksu.edu.sa

Original article

Synergistic activity of colistin in combination with resveratrol and capsaicin against *mcr-1*-posotive *Escherichia coli*

Ping Cheng [a], Botao Wang [a,1], Shuying Liang [a], Yuqi Yang [b], Shixin Gui [a], Kai Zhang [a], Yingying Sun [a], Shaoqi Qu [a,**], Lin Li [a,*]

[a] *Animal-Derived Food Safety Innovation Team, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China*
[b] *School of Basic Medicine, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China*

ARTICLE INFO

Keywords:
Mcr-1
Escherichia coli
Colistin
Resveratrol
Capsaicin
Antibiotic adjuvant

ABSTRACT

Colistin used to be regarded as the last-resort treatment for the infection caused by multidrug-resistant gram-negative bacteria. However, the emergence and widespread of *mcr-1* seriously reduce the clinical effectiveness of colistin, constituting a serious threat to global public health. Due to the difficulties in the development of new antibiotics, restoring antibiotic susceptibility with adjuvants is undoubtedly a rational option. As naturally occurring compounds, resveratrol and capsaicin could be extracted from many natural plants, due to various antimicrobial properties, they have received significant attention. Herein, the synergistic activity of colistin combined with resveratrol and capsaicin against *mcr-1*-positive *Escherichia coli* were investigated by checkerboard method and time-killing assays. The in-depth molecular mechanisms were elucidated by scanning electron microscopy, fluorescent probe experiments, transcriptome and metabolome analysis. Molecular docking assay was taken to analyse potential interactions between resveratrol/capsaicin and MCR-1. Finally, the *in vivo* efficacy of combined therapy against *mcr-1*-positive *Escherichia coli* was assessed. Our results demonstrated that colistin combined with resveratrol and capsaicin acted synergistic activity against *mcr-1*-positive *Escherichia coli* both *in vivo* and *in vitro*. Further mechanistic studies showed that the combined therapy could exacerbate cell membrane damage, increase membrane permeability, disrupt the homeostasis of PMF, inhibit ATP synthesis, and efflux pump activity. In addition, the combined therapy could inhibit central carbon metabolism, and reduce tricarboxylic acid cycle and oxidative phosphorylation. Moreover, molecular docking assay revealed resveratrol/capsaicin could bind to MCR-1 stably. Our study indicated that colistin in combination with resveratrol and capsaicin as a novel therapy could provide a trustworthy foundation to establish the treatment plan for *mcr-1*-positive *Escherichia coli.*

1. Introduction

The antibiotic resistance crisis has become a significant challenge for the global community. Fewer antibiotics are effective in clinical treatment due to the spread of multidrug-resistant bacteria (MDRB) (Li et al., 2023). In case of the difficulty of developing new antibiotics has prompted researchers to explore alternative antimicrobial therapies that diverge from traditional antibiotics (Konwar et al., 2022). Colistin was previously regarded as the ultimate defense against infections caused by multidrug-resistant Gram-negative bacteria (MDRGNB). However, the plasmid-mediated transferable colistin resistance gene *mcr-1* was first identified in *Escherichia coli* isolates from food animals in 2015 (Andrade et al., 2020). The *mcr-1* encoded pEtN transferase which could alter the structure of lipopolysaccharides, particularly lipid A, resulting in colistin resistance (Sherry and Howden 2018). Inevitably, the emergence of transferable resistance gene accelerates the spread of colistin resistance. Now *mcr-1* has been found globally, triggering a new crisis of antibiotic resistance. In the case of difficulties in developing new

* Corresponding author at: Animal-Derived Food Safety Innovation Team, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China.
** Corresponding author at: Animal-Derived Food Safety Innovation Team, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China. Anhui Agricultural University, Hefei 230036, China.
E-mail addresses: sqq@ahau.edu.cn (S. Qu), lilin@ahau.edu.cn (L. Li).
[1] The author contributed equally as the first author.

https://doi.org/10.1016/j.arabjc.2024.105988
Received 17 June 2024; Accepted 2 September 2024
Available online 5 September 2024

antibiotics and the widespread of MDRB, antibiotic adjuvants are considered to be an effective means to overcome antibiotic resistance (Liu et al., 2020a, Okdah et al., 2018).

The natural polyphenol resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a kind of stilbenoid derived from natural plant sources, including grapes, oranges, peanuts, and mulberries. Resveratrol has shown the potential for antibacterial, anticancer, antihypertensive, anti-inflammatory, anti-platelet aggregation and cardioprotective activities (Lee et al., 2022). Resveratrol has been used in the prevention and treatment of different diseases, particularly chronic diseases, such as cancer, neurodegenerative diseases, Alzheimer's and metabolic diseases. In addition, the resveratrol shows significant antibacterial effects against *Campylobacter, Listeria, Staphylococcus aureus*, and *Escherichia coli* by inhibiting an electron transport chain (ETC) and F0F1-ATPase, which could affect the cellular energy supply (Zhang et al., 2021). Resveratrol has been reported to improve the effectiveness of aminoglycosides against *Staphylococcus aureus* by inhibiting the ATP synthesis process (Nohr-Meldgaard et al., 2018). In summary, previous studies have shown that resveratrol could inhibit bacterial energy synthesis, and this ability makes us realize its feasibility as an adjuvant to reverse colistin resistance.

Capsaicin (8-methyl-*N*-vanillyl-6-non-enamide) is an alkaloid extracted from the plants of *capsicum* genus, which is known for its neuroleptic analgesic, anti-tumor and antioxidant properties in pharmacological research. Furthermore, it has been found to modulate blood lipids and decrease obesity in mouse models. Owing to its multiple pharmacological properties, capsaicin is widely used in clinical treatments. It has been proven the efflux pump inhibitor CCCP combined with colistin could reverse colistin resistantce in *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *S. maltophiliau* (Ni et al., 2016). Thus, the combination of CCCP and colistin is considered to be an effective means of restoring colistin susceptibility, but it cannot be used in clinical practice due to its toxicity (Periferakis et al., 2023). In contrast to chemically derived efflux pump inhibitors, natural sources are less toxic. Capsaicin was proven to inhibit the activities of various efflux pumps and yield synergistic antimicrobial effects when combined with various antibiotics. Previous studies have shown that capsaicin in combination with ciprofloxacin inhibited the NORA efflux pump of *Staphylococcus aureus* (Kalia et al., 2012). In addition, capsaicin also exerted a significant efflux pump inhibitory effect in *Mycobacterium smegmatis* (Prasch et al., 2019). Therefore, we chose a plant-derived pump inhibitor capsaicin as an adjuvant for safety reasons to restore colistin susceptibility.

With the emergence and prevalence of *mcr-1*, the growing colistin resistance of *Escherichia coli* has become a serious problem, which seriously reduces the clinical effectiveness of colistin for infectious diseases caused by MDRB, threating global human and public health safety. Therefore, it is urgent to find new strategies to overcome MCR-mediated colistin resistance in *Escherichia coli.* Combination of antibiotics and adjuvants represents a promising means to counter the worsening antibiotic resistance crisis. Study on adjuvants has received more attention, nowadays numerous kinds of compounds have been reported. Among these, antimicrobial substances derived from natural plants are considered to be promising. This study focused on the efficacy of resveratrol combined with capsaicin as novel antibiotic adjuvant. The antimicrobial susceptibility testing, checkerboard method, time-killing assays, and animal experiment were performed to determine the *in vitro* and *in vivo* activity of resveratrol combined with capsaicin. The further study of biochemical index, transcriptomic analysis, metabolome analysis, and molecular docking was performed to identify the molecular mechanism. The findings of this study revealed the potential of resveratrol combined with capsaicin as a new colistin adjuvant, which may become a viable therapeutic alternative for combating *mcr-1*-positive *Escherichia coli* infections.

2. Materials and methods

2.1. Bacteria and chemicals

The specific information of strains used in this study could be found in Table S1. All *mcr-1*-positive *Escherichia coli* were confirmed by PCR analysis of 16S rRNA and *mcr-1*. Colistin (99 %, purity), resveratrol (99 %, purity), capsaicin (98 %, purity) and other chemical reagents were purchased from Mackin (Shanghai, China), Aladdin (Shanghai, China) or Solarbio Technology (Beijing, China).

2.2. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of colistin, resveratrol and capsaicin against *mcr-1*-positive *Escherichia coli* isolates were determined by the micro-dilution method, according to the CLSI 2023 guideline (Rai et al., 2023). The *Escherichia coli* ATCC 25922 was used as a quality control strain. All tests were performed in triplicate for all strains.

2.3. Checkerboard assays

Synergistic activity of colistin and resveratrol, capsaicin was evaluated by checkerboard assays according to the previous study (Yi et al., 2022). The specific details of experiment could be found in supplementary materials S1.

2.4. Time-killing curves

To further explore the synergistic antimicrobial activity of colistin and antibiotic adjuvants, clinical strain *mcr-1*-positive *Escherichia coli* (HP-144) was selected to construct time-killing curves. The specific details of experiment could be found in supplementary materials S2. Compared with the colistin group, synergy was determined if an over 2 log10 reduction of CFU/mL was observed in the drug combination group.

2.5. The determination of biochemical index

The pretreatment of biochemical index assays were conducted as the following protocols. *Escherichia coli* (HP-144) was incubated to exponential grow phase. Then, bacterial suspensions were centrifuged and re-suspended in buffer with an OD_{600} of 0.5. The fluorescent dye was added according to the instructions of different kits. After 30 min incubation at 37 °C, then the mixed cultures were treated with different groups of drugs. After being incubated at 37 °C for 1 h, 200 μL cultures were added to the 96-well plate and the microplate reader was used to evaluate biochemical indexes. The specific excitation wavelength and emission wavelength used in this study were listed in supplementary materials.

2.5.1. Outer membrane permeability assay

The outer membrane was detected by using 1-N-phenylnaphthylamine (NPN) (10 μM) as the indicator. The specific details of experiment could be found in supplementary materials S3.

2.5.2. Cell membrane permeability assay

The cell membrane permeability was detected by using Propidium Iodide (PI) (15 μM)as the indicator. The specific details of experiment could be found in supplementary materials S4.

2.5.3. Intracellular ATP

Intracellular ATP was detected using the Enhanced ATP Assay Kit (Beyotime, Shanghai, China) according to the instruction, the specific step could be found in supplementary materials S5.

2.5.4. ROS level

ROS was detected by using a Reactive Oxygen Species Assay Kit (Beyotime, Shanghai, China) according to the manufacturer's instruction, the specific step could be found in supplementary materials S6.

2.5.5. SOD enzymatic activity

The total Superoxide Dismutase Assay Kit with WST-8 (Beyotime, Shanghai, China) was applied to measure the superoxide dismutase (SOD) activity in bacteria cells.

2.5.6. Efflux pump activity assay

The efflux pump activity was measured by fluorescent probe Ethidium bromide (EB).

2.5.7. Δψ assay

3,3-dipropylthiadicarbocyanine iodide (DiSC3(5), 0.5 μM) was used to determine the membrane potential (Δψ). The specific details of experiment could be found in supplementary materials S7.

2.5.8. ΔpH assay

Fluorescence probe BCECF-AM was used to determine intracellular pH. The specific details of experiment could be found in supplementary materials S8.

2.6. Scanning electron microscopy (SEM)

Escherichia coli HP-144 was grown to the exponential phase, then bacteria were washed, and suspended in LB with OD_{600} of 0.5. Bacterial suspension incubated with drugs for 24 h at 37 °C. Cultures were fixed with 2.5 % glutaraldehyde at 4 °C for 12 h. The bacteria were dehydrated with gradient ethanol (30 %, 50 %, 70 %, 90 %, and 100 %) and precipitates were suspended with acetone at 4 °C and dried at the critical point as previous described (Koh et al., 2022), followed by the observation under the SEM.

2.7. Transcriptomic analysis

The *Escherichia coli* HP-144 in early-exponential phase was treated with colistin (2 μg/mL) alone or in combination with resveratrol (128 μg/mL) and capsaicin (256 μg/mL) for 4 h. Total RNA was extracted by TRIzol Reagent (Invitrogen) according to instructions. The transcriptomic sequencing was performed as previous described, the specific details could be found in supplementary materials S9.

2.8. Metabolome analysis

Escherichia coli HP-144 was grown to the exponential phase, then the bacterial cultures were incubated with colistin (2 μg/mL) alone or in a combination of resveratrol (128 μg/mL) and capsaicin (256 μg/mL) for 4 h. A mass spectrometry (MS)-based metabolomics was performed, the specific details could be found in supplementary materials S10.

2.9. Mouse peritonitis-sepsis infection model

Four-week-old ICR female mice (approximately 20 g) were randomly divided into seven groups (n = 12): Control, CAP (2 mg/kg), RES (50 mg/kg), COL (10 mg/kg), COL+RES (10 mg/kg + 50 mg/kg), COL+CAP (10 mg/kg + 2 mg/kg), COL+RES+CAP (10 mg/kg + 50 mg/kg + 2 mg/kg). ICR female mice were treated with 5.0×10^6 CFUs HP-144 via intraperitoneal injection. Mice were treated with different groups of drugs 2 h after injection. After 48 h of infection, mice were euthanized and the spleen, liver, and kidney were isolated, weighed, homogenized, and serially diluted in PBS. Samples were ten-fold serially diluted and incubated on LB agar plates at 37 °C for 18 h. Bacterial colonies were counted and the primary CFUs/mL were calculated. Moreover, part of the tissues (spleen, liver, and kidney) were subjected to histological analysis.

2.10. Molecular docking

To predict the possible interaction between resveratrol, capsaicin, and MCR-1, molecular docking was performed as previously described (Lee et al., 2022), the specific details could be found in supplementary materials S11.

2.11. Statistical analyses

Statistical analysis was performed using GraphPad Prism 8 8. All datas were presented as mean ± SD. Statistical assessments were performed using unpaired 2-tailed t-tests, one-way ANOVA among multiple groups. Significance levels are indicated with asterisks: *P<0.05, **P<0.01, ***P<0.001.

3. Results

3.1. Synergistic activity of colistin in combination with resveratrol and capsaicin against mcr-1-positive Escherichia coli

Resveratrol or capsaicin was tested against *mcr-1*-positive *Escherichia coli*. As shown in Table 1, the MIC values of 1024 or 2048 μg/mL for resveratrol or capsaicin; sub-inhibitory concentration showed that capsaicin or resveratrol alone had no significant antibacterial activity against *mcr-1*-positive *Escherichia coli*. Resveratrol at a subinhibitory concentration (1/4 MIC, 1/8 MIC, 1/16 MIC) combined with colistin enhanced the activity of colistin, and the MIC fold change of colistin ranges from 4 to 512. Capsaicin at sub-inhibitory concentration (1/4 MIC, 1/8 MIC, 1/16 MIC) combined with colistin enhanced the activity of colistin, and the MIC fold change of colistin ranges from 64 to 2048.

The synergistic activity of colistin in combination with antibiotic adjuvants was evaluated by checkboard assays. As shown in Table 2, the FICI values for resveratrol combined with colistin were from 0.09375 to 0.5. The FICI values for capsaicin combined with colistin were from 0.078125 to 0.15625. This demonstrated that resveratrol and capsaicin had synergistic effects on *mcr-1*-positive *Escherichia coli* when combined with colistin. To further assess the reversal ability of resveratrol and capsaicin in combination with colistin. Antibacterial tests were carried out with 1/16 MIC RES+1/4MIC CAP, 1/16 MIC RES+1/8 MIC CAP, and 1/16 MIC RES+1/4 MIC CAP. The MICs of colistin decreased to ≤ 0.00003 μg/mL, and the colistin MIC fold change ≥ 65536. The combination therapy completely reversed the colistin resistance of all *mcr-1*-positive *Escherichia coil.*

3.2. Time–kill results for the colistin in combination with resveratrol and capsaicin against mcr-1-positive Escherichia coli

Subsequently, the time-kill experiment was carried out on the selected clinical *mcr-1*-positive *Escherichia coli* HP-144 isolated from fecal sample during the exponential growth phase with drugs. As shown in Fig. 1, resveratrol, capsaicin, and colistin alone did not kill exponentially growing *mcr-1*-positive *Escherichia coli* HP-144. Colistin in combination with resveratrol and capsaicin completely killed the bacteria throughout 12–24 h. At 24 h, compared with the colistin alone the bacterial concentration reduced by 11.519 $\log_{10}$ CFU/mL. These results illustrated that the combination of colistin, resveratrol and capsaicin exhibited synergistically to *mcr-1*-positive *Escherichia coli*.

3.3. Morphological changes of mcr-1-positive Escherichia coli after treatment of colistin in combination with resveratrol and capsaicin

Colistin in combination with resveratrol and capsaicin exhibited significant synergistic antibacterial effect. Therefore, we speculate that the combination of colistin, resveratrol and capsaicin treatment could

Table 1
Susceptibility of colistin alone and in combination with resveratrol/capsaicin against 8 isolates of *mcr-1*-positive *Escherichia coli* by microdilution assay.

Strains		RES Microdilution assay(mg/L)			CAP Microdilution assay(mg/L)			RES and CAP Microdilution assay(mg/L)	
	MIC of COL	MIC of RES	MIC of 1/16 RES+COL	MIC fold change	MIC of CAP	MIC of 1/4 CAP+COL	MIC fold change	MIC of 1/16 RES+1/4 CAP+COL	MIC fold change
HP-18	4	1024	0.5	8	1024	0.015625	256	≤0.00003	≥131072
HZ-46	2	1024	0.125	16	1024	0.03125	64	≤0.00003	≥65536
HZ-158	4	2048	0.25	16	1024	0.002	2048	≤0.00003	≥131072
HP-63	2	2048	0.125	16	2048	0.008	256	≤0.00003	≥65536
HP-144	4	2048	1	4	2048	0.03125	128	≤0.00003	≥131072
HP-175	4	2048	1	4	1024	0.03125	128	≤0.00003	≥131072
226	2	2048	0.5	4	1024	0.002	1024	≤0.00003	≥65536
HP-418	4	1024	1	4	1024	0.004	1024	≤0.00003	≥131072

Table 2
FICI of colistin in combination with resveratrol or capsaicin against clinical strains of *mcr-1*-positive *Escherichia coli.*

Strains	COL+RES			COL+CAP		
	MIC (COL/RES)	FICI	Interaction	MIC (COL/CAP)	FICI	Interaction
HP-18	1/256	0.5	synergy	0.5/32	0.15625	synergy
HZ-46	0.125/128	0.1875	synergy	0.25/32	0.15625	synergy
HZ-158	0.125/64	0.09375	synergy	0.5/32	0.15625	synergy
HP-63	1/64	0.53125	additive	0.25/32	0.15625	synergy
HP-144	0.25/256	0.1875	synergy	0.25/32	0.078125	synergy
HP-175	0.25/64	0.09375	synergy	0.5/32	0.15625	synergy
226	0.25/64	0.09375	synergy	0.25/32	0.15625	synergy
HP-418	0.5/32	0.15625	synergy	0.5/64	0.15625	synergy

Note: MIC (COL/RES) is the MIC of colistin in combination with resveratrol. MIC (COL/CAP) is the MIC of colistin in combination with capsaicin. Synergy is defined as an FIC index of ≤0.5.

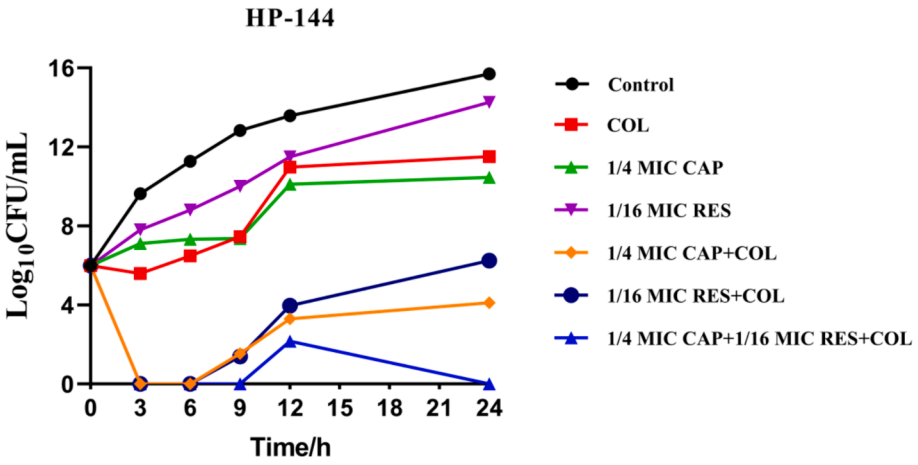

Fig. 1. Time-kill curves of the *Escherichia coli* HP-144 in the presence of resveratrol (128 μg/mL), capsaicin (256 μg/mL) or colistin (2 μg/mL) alone or in combination for 24 h.

promote the destruction of the OM. In order to confirm our hypothesis, SEM was utilized to observe the morphological alterations of *mcr-1*-positive *Escherichia coli* HP-144 after different treatment. As shown in Fig. 2A, the membrane damage and morphological shrinkage caused by combined therapy were more severe compared with mono-treatment. These findings suggested that the colistin combined with resveratrol and capsaicin combination might enhance ability to damage membrane structures.

3.4. Colistin in combination with resveratrol and capsaicin disrupts the bacterial membrane

To visually show the effect of the combined therapy on cell membrane permeability, the OM and IM permeability were measured by NPN and PI, respectively. After the colistin in combination with resveratrol and capsaicin treatment, the fluorescence intensity of NPN increased significantly (Fig. 2B), indicating that the combination observably improved the permeability of the OM which was consistent with the electron microscopy results. In addition, there was no significant change in the permeability of the IM, indicating the integrity of the IM structure was maintained (Fig. 2C). Collectively, the findings demonstrated that

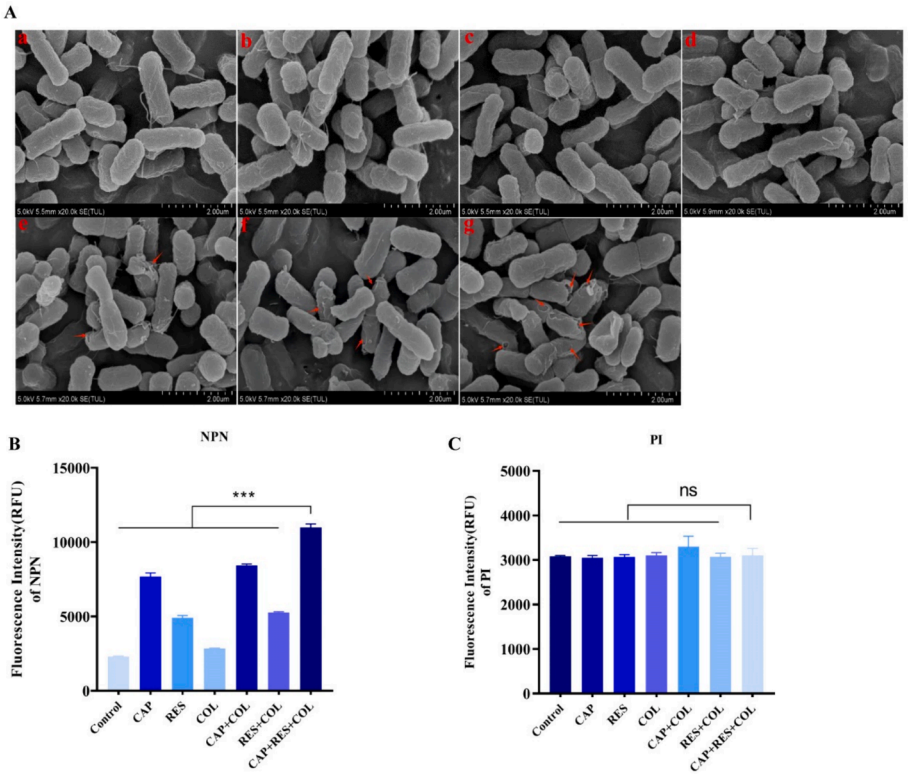

Fig. 2. Effects of combined therapy on bacterial membrane. A, Morphological changes of *Escherichia coli* HP-144 treated with colistin (2 μg/mL), resveratrol (128 μg/mL), capsaicin (256 μg/mL) alone or their combination. Scar bar, 2.00 μm. Destroyed outer membrane was marked by red arrows. The experiment is grouped as follows: (a) Control, (b) CAP, (c) RES, (d) COL, (e) CAP+COL, (f) RES+COL, (g) RES+CAP+COL. B, Outer membrane permeability was evaluated by measuring the fluorescence intensity of NPN. C, Inner membrane permeability was evaluated by measuring the fluorescence intensity of PI.

capsaicin and resveratrol improved colistin's capacity to damage the OM.

3.5. Colistin in combination with resveratrol and capsaicin dissipates the proton motive force

PMF (proton motive force), comprises two components: electric potential (ΔΨ) and transmembrane proton gradient (ΔpH). Due to the two components compensating for each other, the PMF remains stable in the absence of excessive external disturbances. Specifically, to equilibrate the homeostasis of PMF, the ΔpH could increase when the ΔΨ dissipated and vice versa. In fact, the increase of fluorescence intensity of DiSC3(5) suggests the dissipation of ΔΨ, while a decrease otherwise implies the disruption of ΔpH. The results showed that the fluorescence intensity of DiSC3(5) was reduced after treatment (Fig. 3A), which suggested that the drug effect may have affected the change in ΔpH. Therefore, the fluorescence intensity of the fluorescent probe BCECF AM were measured, and we found that the group with the addition of resveratrol had a significant decrease in intracellular pH (Fig. 3B), decreasing ΔpH. Resveratrol was shown to be responsible for the dissipation of ΔpH. The disturbance of PMF by colistin in combination with resveratrol and capsaicin mainly caused the changes of intracellular pH, which disrupted PMF and might affect the related ATP synthesis.

3.6. Colistin in combination with resveratrol and capsaicin inhibits intracellular ATP synthesis of mcr-1-positive Escherichia coli

PMF, as an energy pathway on the cell membrane, can regulate ATP synthesis. For this reason, we further examined the changes of intracellular ATP. As expected, ATP was reduced after drug treatment, while the intracellular ATP level was markedly reduced in *mcr-1*-positive *Escherichia coli* HP-144 treated with the combination (Fig. 3C). Thus, we speculated that the inhibition of ATP production affects the life-related processes of bacteria, which may be related with the enhanced antimicrobial ability of colistin.

3.7. Colistin in combination with resveratrol and capsaicin inhibits efflux pumps activity

In general, the efflux pump plays a vital factor in antimicrobial resistance. As an inhibitor of efflux pump, capsaicin can inhibit the efflux pump activity and ensure the retention of the drug in the cell. In this experiment, it was envisaged that capsaicin could inhibit the efflux pump and increase the intracellular concentration of resveratrol, thereby strengthening the antimicrobial effect of colistin. To prove our assumptions, we measured the activity of the efflux pump, the increase in fluorescence indicates the accumulation of Etbr in the cell, and we found that capsaicin supplementation could increase intracellular accumulation of Etbr (Fig. 3D), indicating that the efflux pump activity was inhibited. These findings demonstrated that colistin in combination with resveratrol and capsaicin could effectively inhibit the efflux pumps activity in *mcr-1*-positive *Escherichia coli*.

3.8. Changes in intracellular oxidation levels after the different treatments

Reactive oxygen species are widely considered to be an essential part of antimicrobial activity. However, controversy remains as to whether reactive oxygen species are the decisive factor for sterilization. It has been reported that bacterial death is not associated with the production of reactive oxygen species. Resveratrol and capsaicin act as exogenous reducing agents that can remove oxidizing substances. Based on the results, we found that the adding resveratrol and capsaicin could reduce the level of ROS. Subsequently, in order to detect the changes in intracellular antioxidant levels, we then measured the main intracellular

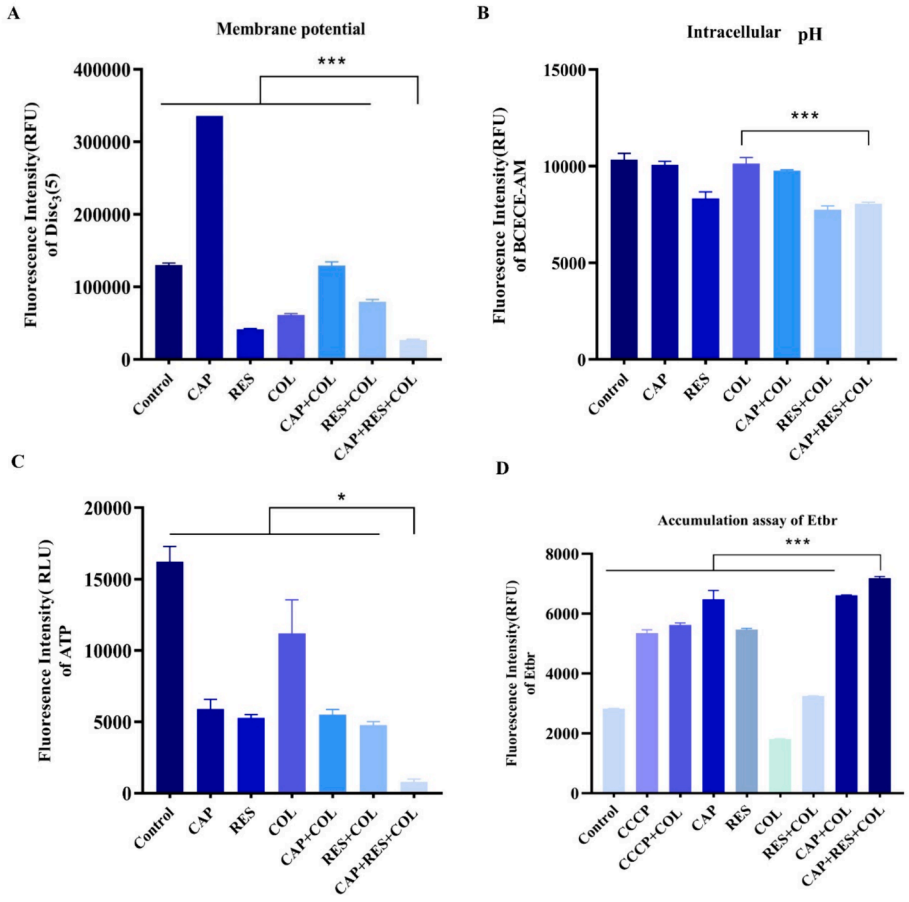

Fig. 3. Effects of combined therapy on bacterial energy metabolism and efflux pump activity in *Escherichia coli* HP-144. A, Intracellular ΔΨ, B, Intracellular pH changes, C, Intracellular ATP levels, D, The activity of efflux pump.

oxidoreductase SOD enzyme, and the activity of SOD enzyme markedly decreased in the *mcr-1*-positive *Escherichia coli* treated with combination of colistin, resveratrol and capsaicin (Fig. 4AB). The results showed that due to the addition of exogenous reducing agents, which were sufficient to remove reactive oxygen species, excessive production of endogenous oxidoreductase was not required. Combined with the results of *in vitro* synergistic activity, it was suggested that the reason for excellent antibacterial effect of resveratrol and capsaicin combined with colistin may not be related to oxidative stress. It may be related to the inhibition of cellular respiration as well as the destruction of cell membranes, but the exact reason remains to be explored.

3.9. Molecular docking

The results of the molecular docking of the MCR-1 protein with bioactive groups of resveratrol and capsaicin are displayed in Fig. 5. The binding free energy between MCR-1 and resveratrol was −5.45 kcal/mol. Resveratrol has the ability to bind to MCR-1 both hydrophobically and through hydrogen bonding. According to the binding model of resveratrol to MCR-1, resveratrol formed strong contacts through hydrogen bonding with VAL413, GLU423, and GLU418 (Fig. 5ABC). Moreover, capsaicin could also bind to MCR-1, the binding free energy between MCR-1 and capsaicin was −3.91 kcal/mol, with hydrogen

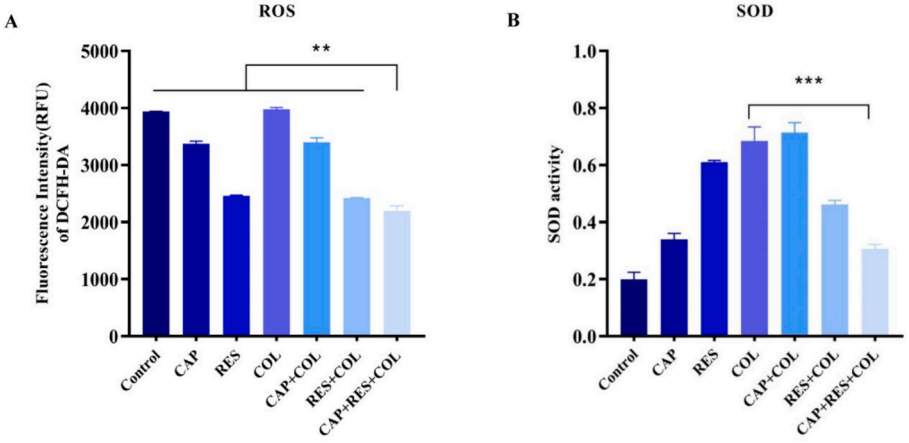

Fig. 4. Oxidative stress of *Escherichia coli* HP-144 after different treatments. A, ROS levels, B, SOD activity.

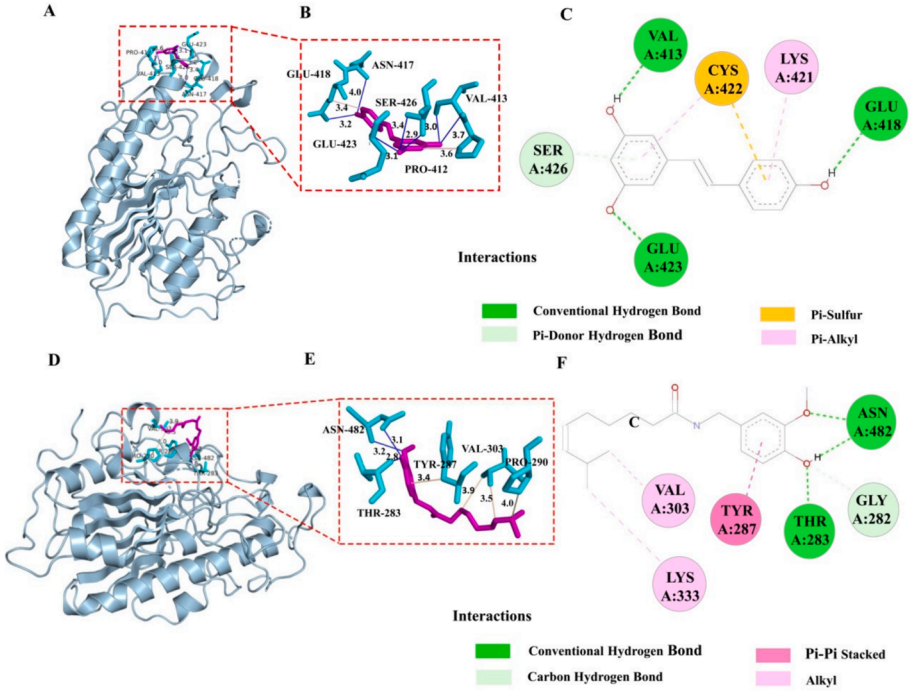

Fig. 5. Putative pattern of interaction between resveratrol, capsaicin and MCR-1 protein. A, Stable 3D structure of resveratrol with MCR-1 obtained from the AutoDock simulations. B, The interactions formed between the amino acid residues (stick) and the docked resveratrol molecule (ball and stick) in the MCR-1 binding site. C, Interaction of planar amino acids between resveratrol and MCR-1 molecule. D, Stable 3D structure of capsaicin with MCR-1 obtained from the - AutoDock simulations. E, The interactions formed between the amino acid residues (stick) and the docked capsaicin molecule (ball and stick) in the MCR-1 binding site. F, Interaction of planar amino acids between capsaicin and MCR-1 molecule.

bonds THR283, ASN482, and TYR287 serving as the primary means of attachment (Fig. 5DEF). In summary, the molecular docking data demonstrated that resveratrol and capsaicin have good bonding stability with the MCR-1 protein.

3.10. Colistin in combination with resveratrol and capsaicin down-regulates TCA cycle and oxidative phosphorylation, inhibited efflux pump, reduced nucleotide metabolism

In order to further investigate the molecular mechanisms of resveratrol and capsaicin enhancing bactericidal activity of colistin against *mcr-1*-positive *Escherichia coli*, we performed transcriptomic and metabonomic analysis of HP-144 isolate after exposure to colistin or colistin combined with resveratrol and capsaicin. The comparison of treatment with the combination and that with colistin alone revealed 1791 differentially expressed genes (DEGs), including 1080 up-regulated genes, and 711 down-regulated genes. KEGG enrichment analysis demonstrated that DEGs are mainly involved in pathways such as TCA cycle, oxidative phosphorylation, and carbon metabolism (Fig. 6). Notably, up-regulated genes involved in oxidation–reduction process, TCA cycle, oxidative phosphorylation and efflux pump system. Similarly, metabolite enrichment analysis demonstrated that differential metabolites mainly including pathways such as nucleotide metabolism, purine metabolism, pyrimidine metabolism, TCA cycle, oxidative phosphorylation, these pathways are closely related to biological life-sustaining processes (Fig. 7). The results showed that the combined therapy mainly inhibited TCA cycle, oxidative phosphorylation, nucleotide metabolism, and efflux pump. The inhibition of the TCA cycle, and oxidative phosphorylation reduced electron transfer and ATP synthesis, and the inhibition of efflux pumps reduced the efflux of drugs, the inhibition of nucleotide metabolism, likewise affecting subsequent intracellular translation and transcription. Redox-related genes indicate a reduced ability of cells to cope with oxidative damage. In summary, we concluded that combined therapy rendered the bacteria imminent death, inhibiting both energy metabolism and essential functions.

3.11. Resveratrol and capsaicin restores colistin activity against mcr-1-positive Escherichia coli HP-144 in vivo

As colistin, resveratrol, and capsaicin showed excellent synergistic antibacterial capacity on *mcr-1*-positive *Escherichia coli in vitro*, we speculated that colistin's therapeutic effectiveness would be regained *in vivo* by reversing MCR-mediated colistin resistance. The efficacy of colistin combined with resveratrol and capsaicin was examined in a mouse model of intraperitoneal infection infected with *mcr-1*-positive *Escherichia coli*. Organ histological analysis of mice showed that the pathological changes caused by bacterial infection in the three-drug group were obviously alleviated relative to the other groups (Fig. 8A). The results showed that the bacterial loads of the liver, spleen, and kidney significantly reduced in the combination of resveratrol, capsaicin and colistin treatment group relative to other groups (Fig. 8B). These results verified that capsaicin and resveratrol significantly restore colistin function *in vivo*.

4. Discussion

The misuse of antibiotics has exacerbated the problem of bacterial resistance, which poses a risk to public and human health. Colistin used to be considered as the last-resort line against MDRGNB. However, the widely use of colistin in the clinic has aggravated colistin resistance, which result in limited and ineffective treatments to infectious diseases caused by MDRGNB. And the emergence of plasmid-mediated mobile colistin resistance (*mcr*) has deepened the need for new effective treatments. Compared to the difficulties in the development of new antibiotics, combination therapy is regarded as an effective antimicrobial means of restoring antibiotic susceptibility. For example, natural flavonoids enhance colistin sensitivity by disrupting the bacteria's iron homeostasis (Zhong et al., 2023). Stilbene acid can restore the

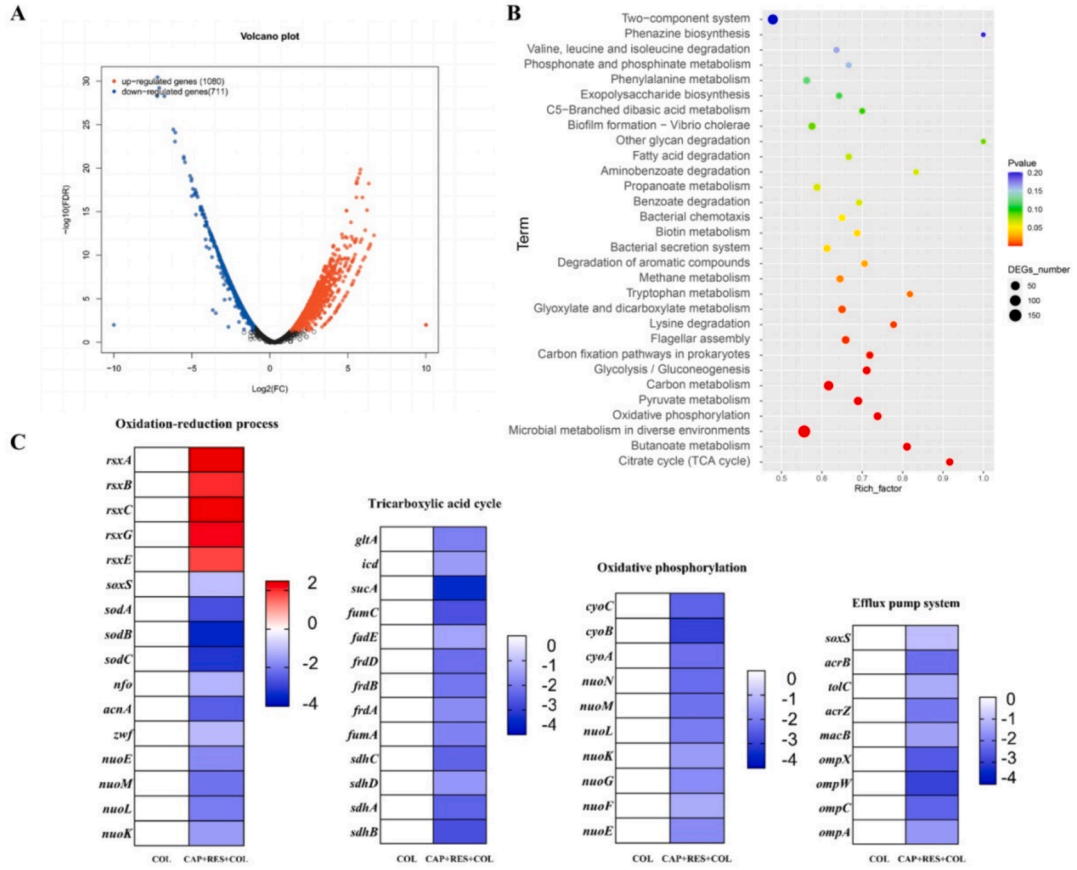

Fig. 6. Transcriptomic analysis of *Escherichia coli* HP-144 treated by colistin, and colistin in combination with resveratrol and capsaicin. A, Volcano-plots of DEGs distribution. B, KEGG enrichment analysis of the DEGs. The x and y axis in A represent the expression changes and corresponding statistically significant degree, respectively. C, Selected DEGs involved in TCA cycle, Oxidative phosphorylation, multidrug efflux pump and oxdation-reduction process. COL, colistin alone, COL+RES+CAP, colistin in combination with resveratrol and capsaicin.

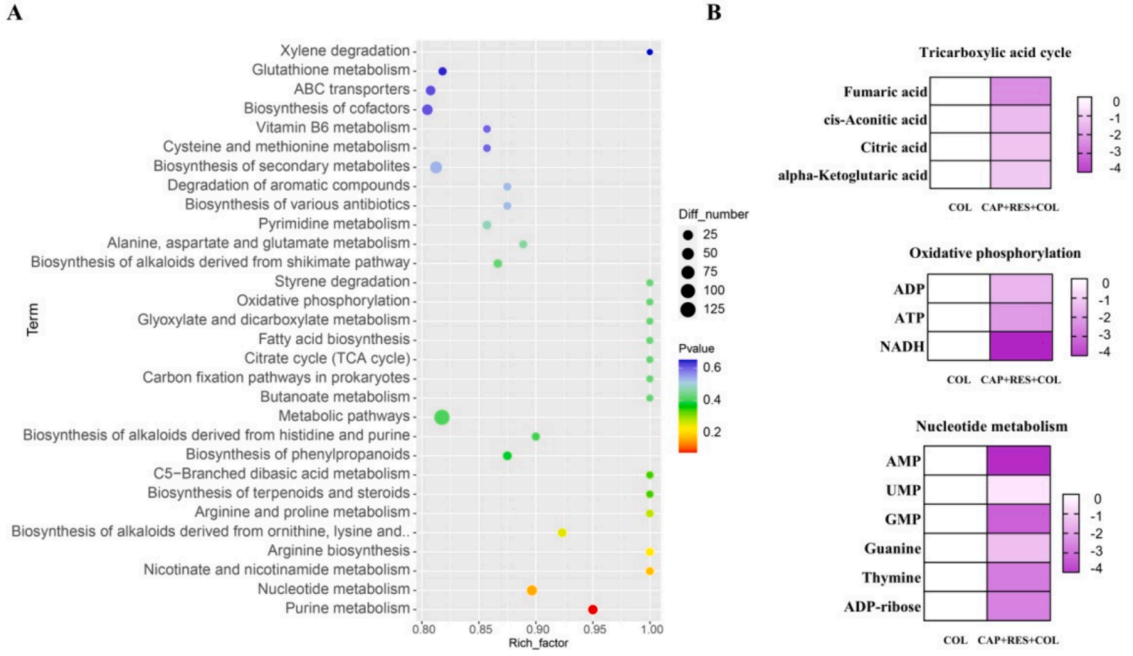

Fig. 7. Non-targeted metabolomics analysis of *Escherichia coli* HP-144 treated by colistin, and colistin in combination with resveratrol and capsaicin. A, KEGG enrichment analysis of the differential metabolites. The x and y axis in A represent the expression changes and corresponding statistically significant degree, respectively. B, Selected differential metabolites involved in TCA cycle, Oxidative phosphorylation, nucleotide metabolites. COL, colistin alone; COL+RES+CAP, colistin in combination with resveratrol and capsaicin.

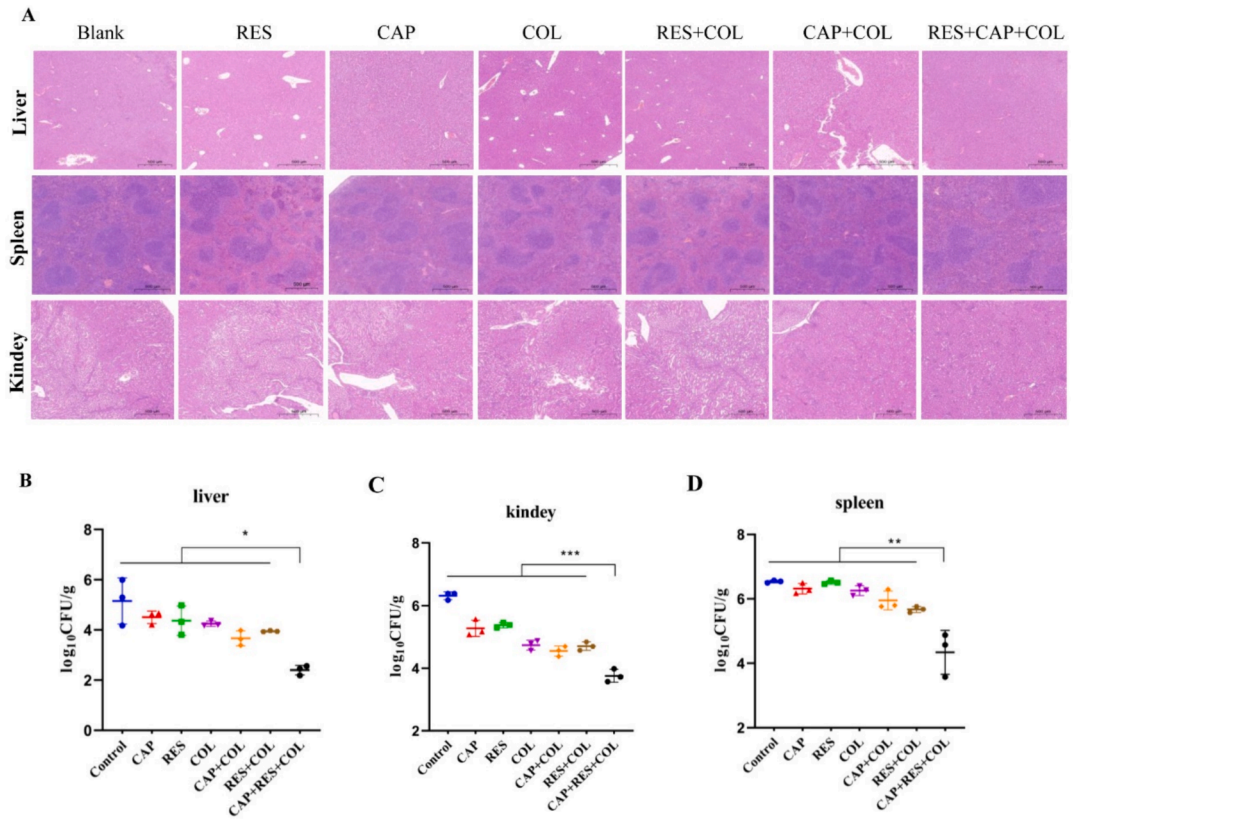

Fig. 8. Resveratrol and capsaicin restore colistin activity in mice infection models. A, Organ histological analysis of mice; B, Bacterial loads were determined in liver; C, Bacterial loads were determined in kidney; D, Bacterial loads were determined in spleen.

sensitivity of polymyxin B by blocking the active site of the MCR-1 protein (Macesic et al., 2017). All of these studies have shown the feasibility of adjuvants in reversing antibiotic resistance.

Anti-inflammatory, antiviral, and antioxidant properties of resveratrol are well-known (Bo et al., 2013, Lima Imperador et al., 2022). Capsaicin is used for topical pain relief, as a cardioprotective measure (Fattori et al., 2016), and in recent years as a efflux pump inhibitor, which has been thought to possess the potential to restore antibiotic resistance. In this study, we demonstrated that the combination of resveratrol and capsaicin could potentiate antibacterial activity of colistin against *mcr-1*-positive *Escherichia coli in vitro* and *in vivo*. The results shows that resveratrol or capsaicin exhibited a potentiation on colistin MIC (4 to 2048-fold change) against *mcr-1*-positive *Escherichia coli*, while the combination of resveratrol and capsaicin showed the highest enhancement effect (colistin MIC fold change $\geq$ 65536-fold) on colistin. Time-kill curve assay also showed that resveratrol and capsaicin significantly increased colistin's antibacterial activity. Moreover, in the mouse peritonitis-sepsis infection model, colistin in combination with resveratrol and capsaicin effectively reduced the bacterial load of the liver, spleen, and kidney. Pathological changes caused by bacterial infection in the combination therapy were significantly alleviated compared with the colistin group. All of our findings showed that the combination of resveratrol and capsaicin increased the potency of colistin against *mcr-1*-positive *Escherichia coli in vitro* and *in vivo* study.

GNB have an intricate barrier called the envelope. Bacterial membranes could regulate the entry and departure of foreign chemicals, which are an important part of their early defenses (Bolla et al., 2011). In other words, the membrane permeability can affect the intracellular concentration of the drug molecule (Schuerholz et al., 2012). In this study, the combination of colistin, resveratrol and capsaicin significantly enhanced the permeability of the OM. Additionally, we observed HP-144 after treated with different drug groups by SEM, combination therapy has resulted in more severe membrane damage and morphological shrinkage compared with the colistin treated alone group. This indicated colistin in combination with resveratrol and capsaicin could effectively destroy the outer membrane permeability, induce membrane cleavage, and lead to bacterial death.

Efflux pumps are the main mechanism that results in antibiotic resistance. The RND efflux pump AcrAB-TolC is considered to be a vital efflux pump related to antibiotic resistance in *Escherichia coli*. Efflux pump inhibitors (EPI). The AcrAB-TolC can be rendered inactive by small molecule inhibitors that specifically target one protein within the AcrB homotrime (Alenazy 2022). In this study, we found that the addition of capsaicin contributed to the accumulation of EtBr in the cell. In addition, the combination of resveratrol and capsaicin reduced the expression of genes associated with the multidrug efflux pump. Efflux pump-related regulatory genes (*soxS*, *acrB*, *tolC*, *macB*) were down-regulated (Duval and Lister 2013). The findings above indicated that the combination of resveratrol and capsaicin could drastically inhibit the efflux pumps activity of *mcr-1*-positive *Escherichia coli.*

Whether the production of ROS is a necessary condition for antibiotic sterilization is still debated in current research. Keren et al found that antibiotics could still exert an antibacterial effect by adding a ROS quencher, thiourea, indicating that the removal of reactive oxygen species did not affect the antibacterial effect (Keren et al., 2013). In addition, Liu et al conducted experiments under anaerobic conditions and found that antibiotics maintain effective action despite the lack of reactive oxygen species production (Liu and Imlay 2013). Resveratrol is a natural nonflavonoid with antioxidative functions and can scavenge ROS (Leonard et al., 2003). Similarly, studies have shown that capsaicin has an antioxidative effect and can scavenge various radical(Kogure et al., 2002). In this study, we found a significant reduction in the fluorescence intensity of DCFH-DA with the addition of resveratrol or capsaicin, which indicated that reactive oxygen species were removed.

Moreover, colistin in combination with resveratrol and capsaicin reduced the expression of the gene for repairing oxidative damage (*nfo*), the gene for reducing oxidizing substances (*sodA*), the gene for synthesizing reducing substances (*acnA*, *zwf*). Combined with the *in vitro* results, the removal of reactive oxygen species does not affect the antibacterial effect. The reason for the excellent antibacterial effect due to other mechanisms, such as inhibition of cellular respiration, and inactivation of efflux pumps.

Bacterial cell membranes have an energy channel called proton motility force (PMF), hypothesised to control ATP production, transduction of signal and synthesis of other macromolecules (Liu et al., 2020b). PMF has been recognized as a novel antimicrobial target due to its regulatory function of numerous critical physiological processes (Yang et al., 2023). It was reported that ML-7 could enhance tigecycline antimicrobial activity by destroying the homeostasis of PMF through rasing ΔpH, resulting in the inhibition of intracellular energy supply, the functions of efflux pump and the production of ROS (Sun et al., 2022). Resveratrol can inhibit the electron transport chain (ETC) and F0F1-ATPase, thereby reducing the generation of cellular energy which is necessary for the transmission of pathogens, and we hypothesize that resveratrol can disrupt proton motility force, and help restore colistin sensitivity. As we expected, combined therapy disrupted the homeostasis of PMF via increasing ΔpH, resulting in reduction of intracellular ATP levels.

Central carbon energy metabolism-PMF is found to be a novel resistance metabolism regulatory mechanism, which reveal that the flow of central carbon metabolites affect the electron transport chain as well as PMF (Meylan et al., 2017, Su et al., 2015, Ye et al., 2018, Yong et al., 2021). Moreover, the repression of macromolecular production such as transcription or translation leads to a decrease in bacterial metabolism. Inhibition of central metabolism might decrease energy consumption, reduce energy expenditure and decelerate the speed of ATP production and cellular respiration (Lobritz et al., 2015). In this study, compared with those treated with colistin alone, genes encoding tricarboxylic acid cycle and oxidative phosphorylation metabolites were significantly repressed by the resveratrol-capsaicin-colistin combination. In addition, metabolomic data also suggested that colistin in combination with resveratrol and capsaicin inhibited tricarboxylic acid cycle pathway metabolites (Fumaric acid, *cis*-Aconitic acid, Citric acid, alpha-Ketoglutaric acid) and oxidative phosphorylation pathway metabolites (ATP, ADP, NADH). This suggested that the combination therapy leaded to a decrease in tricarboxylic acid metabolites, which in turn inhibited the respiratory chain and affected the PMF. As well as, the decrease in nucleotide metabolites pathway (AMP, UMP, GMP) indicates that the transcription or translation of bacteria is inhibited, which leads to a decrease in energy requirements. These results demonstrated that colistin in combination with resveratrol and capsaicin could affect central carbon energy metabolism, inhibit the central metabolism of bacteria, disrupt the homeostasis of PMF, inhibit the energy supply of bacteria, and reduced respiratory efficiency, leading to the bacteria in a condition of impending death.

It has been reported that some natural compound could bind to the MCR-1 active centre, thereby affecting substrate binding and leading to the loss of MCR-1 biological activity. 1-Phenyl-2-(phenylamino) Ethanone derivatives can inhibit protein activity by occupying the cavity of the MCR-1 protein (Lan et al., 2019). Osthole can inactivate biological activity of MCR-1 through binding with MCR-1 and blocking the binding of other substrates with MCR-1 (Zhou et al., 2019). Utilizing a molecular dynamics simulation, the process of the interaction of resveratrol and capsaicin with MCR-1 was examined. The results suggested that resveratrol could bind to the active pocket of MCR-1 (VAL413, GLU423, and GLU418), and capsaicin could bind to the active pocket of MCR-1 (THR283, ASN482, TYR287). These results confirmed that resveratrol and capsaicin could bind to MCR-1 and thereby impair MCR-1′s capacity to bind its substrate, decreasing MCR-1′s biological activity, indicated the mechanisms of reversing colistin resistance from another perspective.

5. Conclusions

In summary, our study showed that the combination of resveratrol and capsaicin exhibited excellent synergistic activity of colistin against *mcr-1*-positive *Escherichia coli* both *in vitro* and *in vivo*. Further study demonstrated that the combined therapy could exacerbate cell membrane damage, increase membrane permeability, inhibit efflux pump activity, affect central carbon energy metabolism, disrupt the homeostasis of PMF, inhibit cellular respiration, and reduce ATP synthesis. Our findings indicated that the combination of resveratrol and capsaicin might be a potential colistin adjuvant, which could provide a new regimen for the treatment of *mcr-1*-positive *Escherichia coli*.

Funding

This work was supported by the Talent research fund project of Anhui Agricultural University (No.rc392109); Scientific research project of colleges and universities in Anhui province (No.2022AH050899, 2023AH051047); the Natural Science Foundation of Anhui Province (No. 2208085MC79, 2308085QC107); the Guizhou Provincial Basic Research Program (Natural Science) (No. Qiankehebasic-ZK [2022] General 501).

CRediT authorship contribution statement

Ping Cheng: Project administration, Funding acquisition, Conceptualization. **Botao Wang:** Writing – original draft, Methodology. **Shuying Liang:** Methodology. **Yuqi Yang:** Formal analysis. **Shixin Gui:** Methodology. **Kai Zhang:** Methodology. **Yingying Sun:** Methodology. **Shaoqi Qu:** Resources, Conceptualization. **Lin Li:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2024.105988.

References

Alenazy, R., 2022. Drug Efflux Pump Inhibitors: A Promising Approach to Counter Multidrug Resistance in Gram-Negative Pathogens by Targeting AcrB Protein from AcrAB-TolC Multidrug Efflux Pump from *Escherichia Coli*. Biology (basel) 11, 1328. https://doi.org/10.3390/biology11091328.

Andrade, F.F., Silva, D., Rodrigues, A., et al., 2020. Colistin Update on Its Mechanism of Action and Resistance. Present and Future Challenges. Microorganisms. 8, 1716. https://doi.org/10.3390/microorganisms8111716.

Bo, S., Ciccone, G., Castiglione, A., et al., 2013. Anti-Inflammatory and Antioxidant Effects of Resveratrol in Healthy Smokers A Randomized, Double-Blind, Placebo-Controlled. Cross-over Trial. Curr Med Chem. 20, 1323–1331. https://doi.org/10.2174/0929867311320100009.

Bolla, J.-M., Alibert-Franco, S., Handzlik, J., et al., 2011. Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. FEBS Lett. 585, 1682–1690. https://doi.org/10.1016/j.febslet.2011.04.054.

Duval, V., Lister, I.M., 2013. MarA, SoxS and Rob of Escherichia coli - Global regulators of multidrug resistance, virulence and stress response. Int J Biotechnol Wellness Ind. 2, 101–124. https://doi.org/10.6000/1927-3037.

Fattori, V., Hohmann, M.S.N., Rossaneis, A.C., et al., 2016. Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses. Molecules. 21, 844. https://doi.org/10.3390/molecules21070844.

Kalia, N.P., Mahajan, P., Mehra, R., et al., 2012. Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of Staphylococcus aureus. J Antimicrob Chemother. 67, 2401–2408. https://doi.org/10.1093/jac/dks232.

Keren, I., Wu, Y., Inocencio, J., et al., 2013. Killing by Bactericidal Antibiotics Does Not Depend on Reactive Oxygen Species. Science 339, 1213–1216. https://doi.org/10.1126/science.1232688.

Kogure, K., Goto, S., Nishimura, M., et al., 2002. Mechanism of potent antiperoxidative effect of capsaicin. Biochim Biophys Acta. 1573, 84–92. https://doi.org/10.1016/S0304-4165(02)00335-5.

Koh, Y. I., K. S. Oh, J. A. Kim, et al., 2022. OSBPL2 mutations impair autophagy and lead hearing loss, potentially remedied by rapamycin. Autophagy. 18,2593-2614.doi: 10.1080/15548627.2022.2040891.

Konwar, A.N., Hazarika, S.N., Bharadwaj, P., et al., 2022. Emerging Non-Traditional Approaches to Combat Antibiotic Resistance. Current Microbiology. 79, 330. https://doi.org/10.1007/s00284-022-03029-7.

Lan, X.-J., Yan, H.-T., Lin, F., et al., 2019. Design, Synthesis and Biological Evaluation of 1-Phenyl-2-(phenylamino) Ethanone Derivatives as Novel MCR-1 Inhibitors. Molecules. 24, 2719. https://doi.org/10.3390/molecules24152719.

Lee, Y.J., Kim, J.K., Jung, C.H., et al., 2022. Chemical modulation of SQSTM1/p62-mediated xenophagy that targets a broad range of pathogenic bacteria. Autophagy. 18, 2926–2945. https://doi.org/10.1080/15548627.2022.2054240.

Leonard, S.S., Xia, C., Jiang, B.H., et al., 2003. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem Biophys. 309, 1017–1026. https://doi.org/10.1016/j.bbrc.2003.08.105.

Li, J., Liu, C., Muller, U., et al., 2023. Autophagy proteins are essential for aminoglycoside-induced hearing loss. Autophagy. 19, 1599–1600. https://doi.org/10.1080/15548627.2022.2127525.

Lima Imperador, C.H., Scarim, C.B., Bosquesi, P.L., et al., 2022. Resveratrol and Curcumin for Chagas Disease Treatment-A Systematic Review. Pharmaceuticals (basel). 15, 609. https://doi.org/10.3390/ph15050609.

Liu, Y., Imlay, J.A., 2013. Cell Death from Antibiotics Without the Involvement of Reactive Oxygen Species. Science. 339, 1210–1213. https://doi.org/10.1126/science.1232751.

Liu, Y., Jia, Y., Yang, K., et al., 2020a. Melatonin overcomes MCR-mediated colistin resistance in Gram-negative pathogens. Theranostics. 10, 10697–10711. https://doi.org/10.7150/thno.45951.

Liu, Y., Jia, Y.Q., Yang, K.N., et al., 2020b. Metformin Restores Tetracyclines Susceptibility against Multidrug Resistant Bacteria. Adv Sci. 7, 1902227. https://doi.org/10.1002/advs.201902227.

Lobritz, M.A., Belenky, P., Porter, C.B.M., et al., 2015. Antibiotic efficacy is linked to bacterial cellular respiration. Proc Natl Acad Sci U S A 112, 8173–8180. https://doi.org/10.1073/pnas.1509743112.

Macesic, N., Green, D., Wang, Z., et al., 2017. Detection of *mcr-1*-Carrying *Escherichia coli* Causing Bloodstream Infection in a New York City Hospital: Avian Origins, Human Concerns? Open Forum Infect Dis. 4, 115. https://doi.org/10.1093/ofid/ofx115.

Meylan, S., Porter, C.B.M., Yang, J.H., et al., 2017. Carbon Sources Tune Antibiotic Susceptibility in Pseudomonas aeruginosa via Tricarboxylic Acid Cycle Control. Cell Chem Biol. 24, 195–206. https://doi.org/10.1016/j.chembiol.2016.12.015.

Ni, W., Li, Y., Guan, J., et al., 2016. Effects of Efflux Pump Inhibitors on Colistin Resistance in Multidrug-Resistant Gram-Negative Bacteria. Antimicrob Agents Chemother. 60, 3215–3218. https://doi.org/10.1128/aac.00248-16.

Nohr-Meldgaard, K., Ovsepian, A., Ingmer, H., et al., 2018. Resveratrol enhances the efficacy of aminoglycosides against Staphylococcus aureus. Int J Antimicrob Agents. 52, 390–396. https://doi.org/10.1016/j.ijantimicag.2018.06.005.

Okdah, L., Le Page, S., Olaitan, A.O., et al., 2018. New therapy from old drugs: synergistic bactericidal activity of sulfadiazine with colistin against colistin-resistant bacteria, including plasmid-mediated colistin-resistant *mcr-1* isolates. Int J Antimicrob Agents. 51, 775–783. https://doi.org/10.1016/j.ijantimicag.2018.01.027.

Periferakis, A.-T., Periferakis, A., Periferakis, K., et al., 2023. Antimicrobial Properties of Capsaicin: Available Data and Future Research Perspectives. Nutrients 15, 4097. https://doi.org/10.3390/nu15194097.

Prasch, S., Duran, A.G., Chinchilla, N., et al., 2019. Resistance modulatory and efflux-inhibitory activities of capsaicinoids and capsinoids. Bioorg Chem. 82, 378–384. https://doi.org/10.1016/j.bioorg.2018.10.062.

Rai, S., Dash, D., Agarwal, N., 2023. Introducing the new face of CLSI M100 in 2023: An explanatory review. Indian J Med Microbiol. 46, 100432 https://doi.org/10.1016/j.ijmmb.2023.100432.

Schuerholz, T., Dömming, S., Hornef, M., et al., 2012. Bacterial Cell Wall Compounds as Promising Targets of Antimicrobial Agents II. Immunological and Clinical Aspects. Curr Drug Targets. 13, 1131–1137. https://doi.org/10.2174/138945012802002438.

Sherry, N., Howden, B., 2018. Emerging Gram negative resistance to last-line antimicrobial agents fosfomycin, colistin and ceftazidime-avibactam – epidemiology, laboratory detection and treatment implications. Expert Rev Anti Infect Ther. 16, 289–306. https://doi.org/10.1080/14787210.2018.1453807.

Su, Y.-B., Peng, B., Han, Y., et al., 2015. Fructose Restores Susceptibility of Multidrug-Resistant Edwardsiella tard to Kanamycin. J Proteome Res. 14, 1612–1620. https://doi.org/10.1021/pr501285f.

Sun, L.L., Sun, L., Li, X., et al., 2022. A Novel Tigecycline Adjuvant ML-7 Reverses the Susceptibility of Tigecycline-Resistant Klebsiella pneumoniae. Front Cell Infect Microbiol. 11, 809542 https://doi.org/10.3389/fcimb.2021.809542.

Yang, B., Tong, Z., Shi, J., et al., 2023. Bacterial proton motive force as an unprecedented target to control antimicrobial resistance. Med Res Rev. 43, 1068–1090. https://doi.org/10.1002/med.21946.

Ye, J.-Z., Lin, X.-M., Cheng, Z.-X., et al., 2018. Identification and efficacy of glycine, serine and threonine metabolism in potentiating kanamycin-mediated killing of Edwardsiella piscicida. J Proteomics. 183, 34–44. https://doi.org/10.1016/j.jprot.2018.05.006.

Yi, K., Liu, S., Liu, P., et al., 2022. Synergistic antibacterial activity of tetrandrine combined with colistin against MCR-mediated colistin-resistant Salmonella. Bimed Pharmacother. 149, 112873 https://doi.org/10.1016/j.biopha.2022.112873.

Yong, Y., Zhou, Y.H., Liu, K.X., et al., 2021. Exogenous Citrulline and Glutamine Contribute to Reverse the Resistance of Salmonella to Apramycin. Front Microbiol. 12, 759170 https://doi.org/10.3389/fmicb.2021.759170.

Zhang, L.-X., Li, C.-X., Kakar, M.U., et al., 2021. Resveratrol (RV): A pharmacological review and call for further research. Bimed Pharmacother. 143, 112164 https://doi.org/10.1016/j.biopha.2021.112164.

Zhong, Z.-X., Zhou, S., Liang, Y.-J., et al., 2023. Natural flavonoids disrupt bacterial iron homeostasis to potentiate colistin efficacy. Sci Adv. 9, eadg4205. https://doi.org/10.1126/sciadv.adg4205.

Zhou, Y., Wang, J., Guo, Y., et al., 2019. Discovery of a potential MCR-1 inhibitor that reverses polymyxin activity against clinical *mcr-1*-positive Enterobacteriacea. J Infect. 78, 364–372. https://doi.org/10.1016/j.jinf.2019.03.004.