**Supplementary materials**

**S1. Checkerboard assays**

Synergistic activity of colistin and resveratrol, capsaicin was evaluated by checkerboard assays with two-fold serial dilution of drugs (8×8 matrix). Colistin was diluted along the abscissa while resveratrol or capsaicin was diluted along the ordinate. After being incubated with bacterial suspension (1.0×106 CFU/mL) at 37°C for 18 h, MICs were recorded, and the fractional inhibitory concentration index (FICI) was calculated as follows.

FIC index=FICIa+FICIb=MICab/MICa+MICba/MICb.

MICa is the MIC of compound A alone, MICab the MIC is of compound A in combination with compound B, MICb is the MIC of compound B alone, MICba is the MIC of compound B in combination with compound A, and FICa is the FIC of compound A and FICb is the FIC of compound B. Synergy is defined as FIC index of ≤0.5.

**S2. Time-killing curves**

To further explore the synergistic antimicrobial activity of colistin and antibiotic adjuvants, clinicalstrain *mcr-1*-positive *Escherichia coli* (HP-144) was selected to construct time-killing curves. First, the HP144 was incubated to exponential period. Then, bacteria were treated with different groups of drugs: 1/2 MIC colistin, 1/4 MIC capsaicin, 1/16MIC resveratrol, 1/4 MIC capsaicin+1/2 MIC colistin, 1/16 MIC resveratrol+1/2 MIC colistin, 1/4 MIC capsaicin+1/16 MIC resveratrol+1/2 MIC colistin, negative control (LB). After being cultured at 37°C with 180 rpm for 24 h, 100 µL aliquots were extracted at 0 h, 3 h, 6 h, 9 h, 12 h, and 24 h, and then re-suspended in PBS after centrifugation. After incubation at 37°C for 18 h, the bacterial solution was diluted and spread onto LB agar plates. Colonies were counted and CFUS was calculated for subsequent time-killing curves profiling. All experiments were replicated three times. Compared with the colistin group, synergy was determined if an over 2 log10 reduction of CFU/mL was observed in the drug combination group.

**S3.** **Outer membrane permeability assay**

The outer membrane was detected by using 1-N-phenylnaphthylamine (NPN) (10 μM)as the indicator. Fluorescence intensity was measured by a Microplate reader with the excitation wavelength at 350 nm and the emission wavelength at 420 nm.

**S4. Cell membrane permeability assay**

The cell membrane permeability was detected by using Propidium Iodide (PI) (15 μM)as the indicator. Fluorescence intensity was measured by a Microplate reader with the excitation wavelength at 535 nm and the emission wavelength at 615 nm.

**S5. Intracellular ATP**

Intracellular ATP was detected using the Enhanced ATP Assay Kit (Beyotime, Shanghai, China). The bacterial suspension was incubated with drugs for 30 min, bacterial cultures were centrifuged and the supernatant was removed. Bacterial precipitates were lysed by lysozyme, and the supernatant was prepared for intracellular ATP levels measurement. The detecting solution was added to a 96-well plate and incubated at room temperature for 5 min. Subsequently, the luminescence of supernatant was monitored by a Spark 10 M Microplate reader (Tecan).

**S6. ROS level**

ROS was detected by using a Reactive Oxygen Species Assay Kit (Beyotime, Shanghai, China). Fluorescence intensity was measured with the excitation wavelength at 488 nm and emission wavelength at 525 nm.

**S7. Δψ assay**

3,3-dipropylthiadicarbocyanine iodide (DiSC3(5), 0.5 μM) (Aladdin, Shanghai, China) was applied to determine the membrane potential (Δψ) Fluorescence intensity was measured with the excitation wavelength at 622 nm and emission wavelength at 670 nm.

**S8. ΔpH assay**

Fluorescence probe BCECF-AM was used to determine intracellular pH. Fluorescence intensity was measured with the excitation wavelength at 500 nm and emission wavelength at 522 nm.

**S9. Transcriptomic analysis**

Early-exponential phase *Escherichia coli* HP-144 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 were performed in the following process, including the RNA extraction, library preparation, and Illumina Hiseq sequencing, reads quality control, differential expression analysis and functional enrichment annotation. To identify DEGs (differential expression genes) between the two groups, the expression level for each transcript was calculated using the fragments per kilobase of read per million mapped reads (RPKM) method. edgeR (https://bioconductor.org/packages/release/bioc/html/edgeR.html) was used for differential expression analysis. The DEGs between two groups were selected using the following criteria: i) the logarithmic of fold change was greater than 2 and the false discovery rate (FDR) should be less than 0.05. To understand the functions of the differential expressed genes, GO functional enrichment and KEGG pathway analysis were carried out by Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do) respectively. DEGs were significantly enriched in GO terms and metabolic pathways when their Bonferroni-corrected P-value was less than 0.05.

**S10. Metabolome analysis**

*Escherichia coli* HP-144 was grown to 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 as previously described. Briefly, metabolites of samples were extracted and LC-MS/MS analysis was conducted. Metabolites were annotated using the KEGG database (<https://www.genome.jp/kegg/pathway.html),> HMDB database (https://hmdb.ca/metabolites) and LIPID Mapsdatabase (http://www.lipidmaps.org/). Principal components analysis (PCA) and

Partial least squares discriminant analysis (PLS-DA) were performed at metaX (aflexible and comprehensive software for processing metabolomics data). We appliedunivariate analysis (t-test) to calculate the statistical significance (P-value). The metabolites with VIP > 1 and P-value< 0.05 and fold change≥ 2 or FC≤ 0.5 were considered to be differential metabolites.

**S11. Molecular docking**

To study the possible interaction between resveratrol, capsaicin, and MCR-1, molecular docking was performed via Autodock software. The Crystal structure of MCR-1 was available at the Protein Data Bank in PDB format. The structure of resveratrol and capsaicin was obtained from the PubChem Project. The Discovery Studio system molecular graphics were used to analyze their interaction patterns with binding site residues.

Table S1 The specific information of strains used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| Strains | Colistin resistance | MIC of colistin (µg/mL) | The specific source of the strain |
| HP-18 | *mcr-1* | 4 | Swine fecal sample |
| HZ-46 | *mcr-1* | 2 | Swine fecal sample |
| HZ-158 | *mcr-1* | 4 | Swine fecal sample |
| HP-63 | *mcr-1* | 2 | Swine fecal sample |
| HP-144 | *mcr-1* | 4 | Swine fecal sample |
| HP-175 | *mcr-1* | 4 | Swine fecal sample |
| 226 | *mcr-1* | 2 | Swine fecal sample |
| HP-418 | *mcr-1* | 4 | Swine fecal sample |

Table S2 Susceptibility of colistin alone and in combination with resveratrol against 8 isolates of *mcr-1*-positive *Escherichia coli* by micro-dilution assay

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strains | COL  resistance | MICs (μg/mL) | | | | | | |
|  | COL | +1/4 MIC RES | MIC fold | +1/8 MIC RES | MIC fold | +1/16 MIC RES | MIC fold |
|  |  | change | change | change |
| HP-18 | *mcr-1* | 4 | 0.25 | 16 | 0.5 | 8 | 0.5 | 8 |
| HZ-46 | *mcr-1* | 2 | 0.5 | 4 | 0.125 | 16 | 0.125 | 16 |
| HZ-158 | *mcr-1* | 4 | 0.5 | 8 | 0.5 | 16 | 0.25 | 16 |
| HP-63 | *mcr-1* | 2 | 0.5 | 4 | 0.125 | 8 | 0.125 | 8 |
| HP-144 | *mcr-1* | 4 | 0.008 | 512 | 0.0625 | 64 | 1 | 2 |
| HP-175 | *mcr-1* | 4 | 0.25 | 16 | 0.25 | 16 | 1 | 4 |
| 226 | *mcr-1* | 2 | 0.0625 | 32 | 0.125 | 16 | 0.5 | 8 |
| HP-418 | *mcr-1* | 4 | 0.125 | 16 | 0.125 | 16 | 1 | 4 |

Table S3 Susceptibility of colistin alone and in combination with capsaicin against 8 isolates of

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strains | COL  resistance | MICs (μg/mL) | | | | | | |
|  | COL | +1/4 MIC CAP | MIC fold | +1/8 MIC CAP | MIC fold | +1/16 MIC CAP | MIC fold |
|  |  | change | change | change |
| HP-18 | *mcr-1* | 4 | 0.015625 | 256 | 0.015625 | 256 | 0.002 | 2048 |
| HZ-46 | *mcr-1* | 2 | 0.03125 | 64 | 0.015625 | 128 | 0.015625 | 128 |
| HZ-158 | *mcr-1* | 4 | 0.002 | 2048 | 0.002 | 2048 | 0.03125 | 128 |
| HP-63 | *mcr-1* | 2 | 0.008 | 256 | 0.015625 | 128 | 0.03125 | 64 |
| HP-144 | *mcr-1* | 4 | 0.03125 | 128 | 0.03125 | 128 | 0.03125 | 128 |
| HP-175 | *mcr-1* | 4 | 0.03125 | 128 | 0.002 | 2048 | 0.002 | 2048 |
| 226 | *mcr-1* | 2 | 0.002 | 1024 | 0.03125 | 64 | 0.03125 | 64 |
| HP-418 | *mcr-1* | 4 | 0.004 | 1024 | 0.008 | 512 | 0.03125 | 128 |

*mcr-1*-positive *Escherichia coli* by micro-dilution assay

Table S4 Abbreviations and corresponding full names in the article

|  |  |
| --- | --- |
| Abbreviations | Full names |
| MDRB | multidrug-resistant bacteria |
| MDRGNB | multidrug-resistant Gram-negative bacteria |
| *mcr* | mobile colistin resistance |
| ETC | electron transport chain |
| ATP | adenosine triphosphate |
| CCCP | carbonyl cyanide 3-chlorophenylhydrazone |
| MIC | minimum inhibitory concentration |
| FICI | fractional inhibitory concentration index |
| NPN | 1-N-phenylnaphthylamine |
| PI | Propidium Iodide |
| ROS | reactive oxygen species |
| SOD | superoxide dismutase |
| DiSC3(5) | 3,3-dipropylthiadicarbocyanine iodide |
| SEM | Scanning electron microscopy |
| COL | colistin |
| RES | resveratrol |
| CAP | capsaicin |
| CFU/mL | colony-forming units per milliliter |
| OM | outer membrane |
| IM | inner membrane |
| PMF | proton motive force |
| ΔΨ | electric potential |
| ΔpH | transmembrane proton gradient |
| Etbr | ethidium bromide |
| TCA cycle | tricarboxylic acid cycle |
| DEG | differentially expressed gene |
| EPI | efflux pump inhibitors |
| NADH | nicotinamide adenine dinucleotide |
| ADP | adenosine -diphosphate |
| AMP | adenosine monophosphate |
| UMP | uridine monophosphate |
| GMP | guanosine5'-monophos-phate |