**Bavachin rejuvenates sensitivity of colistin against colistin-resistant Gram-negative bacteria**

Jie Li [a, c], Ning Han [a, c], Zhengyuan He [a, b], Xiaolan Dai [a, b], Feifei Zhao [b, c], Yangyang Li [b], Wenguang Xiong [c], Zhenling Zeng [a, c] *

a Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China.

b National Laboratory of Safety Evaluation (Environmental Assessment) of Veterinary Drugs, South China Agricultural University, Guangzhou 510642, China.

c National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, Guangzhou 510642, China.

*Corresponding author: Zhenling Zeng

E-mail: zlzeng@scau.edu.cn

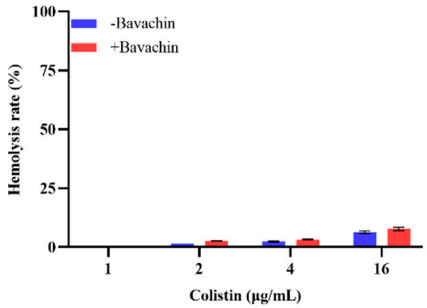


**Figure S1** Hemolytic activity of colistin (1, 2, 4 and 16 μg/mL) in the absence or presence of 32 μg/mL bavachin. Triton X-100 and PBS were used as positive control and negative control, respectively.

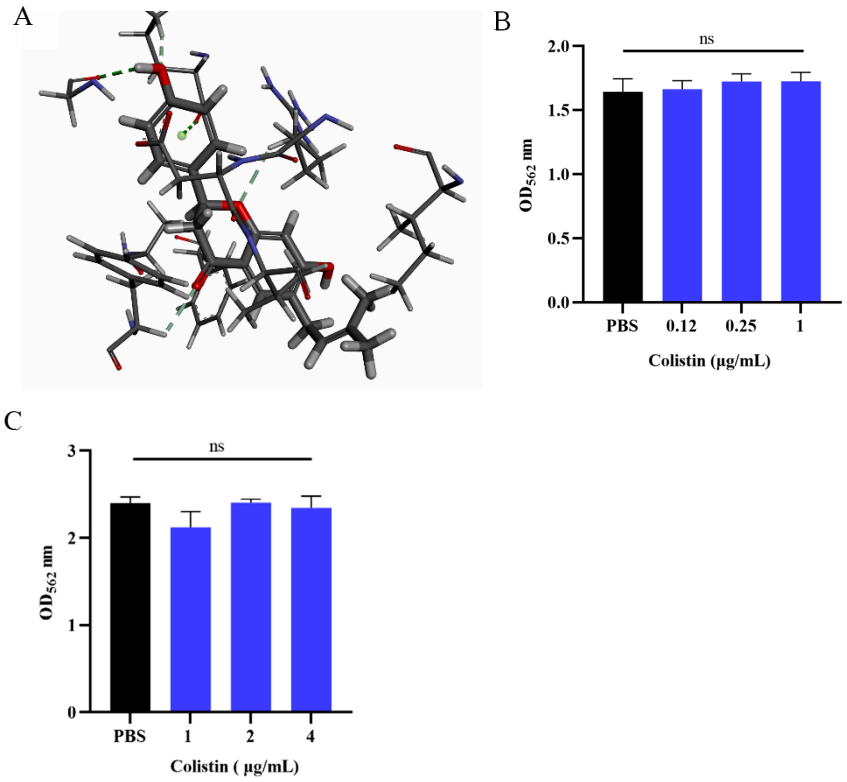


**Figure S2** Synergistic antibacterial mechanism of bavachin combined with colistin against Gram-negative bacteria.

(A) The potential binding mode of bavachin and colistin by molecular docking.

(B) and (C) Bacterial protein leakage of *E. coli* ATCC 25922 (B) and *E. coli* SHP45 (C) after treatment with different concentrations of colistin in the present of 32 μg/mL bavachin for 6 h.

**Table S1** The MIC values of bavachin and colistin against Gram-negative bacteria

| strain | *mcr* gene | MIC (μg/mL) | | FICI |
|---|---|---|---|---|
| | | colistin | bavachin | |
| *E. coli* ATCC25922 | negative | 0.25 | > 128 | 0.125 |
| *E. coli* SHP45 | positive | 4 | > 128 | 0.125 |
| *E. coli* GDQ8D147 | negative | 8 | > 128 | 0.25 |
| *E. coli* GDQ8P27 | positive | 4 | > 128 | 0.25 |
| *P. multocida* CVCC434 | negative | 0.5 | > 128 | 0.125 |
| *K. pneumoniae 212* | negative | 4 | > 128 | 0.125 |
| *K. pneumoniae ATCC700603* | negative | 0.5 | > 128 | 0.25 |
| *S. typhimurium* 26FS14 | positive | 2 | > 128 | 0.125 |
| *S. typhimurium* S226 | positive | 2 | > 128 | 0.125 |
| *S. typhimurium* ATCC14028 | negative | 0.25 | > 128 | 0.25 |

**Table S2** The qRT-PCR primer used in this study

| Primer names | Sequence (5’ to 3’) | Size (bp) |
|---|---|---|
| mcr-1 -F | AAAGACGCGGTACAAGCAAC | 213 |
| mcr-1-R | GCTGAACATACACGGCACAG | |
| 16s- F | TCCTACGGGAGGCAGCAGT | 467 |
| 16s -R | GGACTACCAGGGTATCTAATCCTGTT | |