

Differences in Vivo and in Vitro Antibacterial Activities of Fosfomycin Combined with Amikacin Against Heterogeneous Drug-resistant Escherichia Coli Bloodstream Infections

Linhong Wu¹, Shifeng Huang^{1*}

¹ Department of Laboratory Medicine, the First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Email Address

1131798609@qq.com (Linhong Wu), sfhuang@hospital.cqmu.edu.cn (Shifeng Huang)

*Correspondence: sfhuang@hospital.cqmu.edu.cn

Received: 22 October 2024; Accepted: 13 November 2024; Published: 25 November 2024

Abstract:

The spread of carbapenemase-producing Enterobacteriaceae (CPE) is concerning due to limited treatment options. CPE bloodstream infections (BSIs) have a high mortality rate, and combination therapy could be a potential treatment approach. In this study, a clinically isolated bloodstream infection strain of *Escherichia coli* with heterogeneous resistance to fosfomycin and amikacin was examined. The strain exhibited MIC values of 2mg/L for fosfomycin and 6mg/L for amikacin, with its heterogeneous drug resistance phenotype confirmed using the PAP method. Various methods, including the KB method, E-test, and agar checkerboard dilution method, were employed to validate the superimposed antibacterial activity of the combination treatment. The efficacy of fosfomycin and amikacin alone and in combination was assessed in experimental bloodstream infections in mice over a 24-hour period. Results showed that fosfomycin alone and in combination with amikacin significantly reduced bacterial load in the livers of BSI model mice ($P < 0.05$) compared to the untreated control group, with better outcomes observed than with amikacin alone. However, there was no significant difference in antibacterial activity between the fosfomycin single drug and combined drug groups.

Keywords:

Escherichia Coli, Fosfomycin, Amikacin, Combination Therapy, Blood Stream Infection

1. Introduction

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* has become a common cause of infections in healthcare settings and the community. The CTX-M-15 type enzyme is now the main culprit behind most ESBL-producing *E. coli* infections in Western countries. Carbapenem antibiotics, with their broad antibacterial

activity, are increasingly used to combat these infections, but their efficacy is threatened by the rapid rise of carbapenemase-producing strains [1]. Hence, developing alternative therapies for treating ESBL-producing *E. coli* infections is crucial. The emergence and global spread of ESBL-producing Enterobacteriaceae, including *E. coli*, has led to a significant increase in carbapenem usage. These strains often develop resistance to multiple classes of antibiotics, posing significant treatment challenges. The most effective alternative antibiotics available today are either toxic or less potent, contributing to the high mortality rate associated with CPE infections. Combination antibiotic therapies are now recommended to enhance effectiveness, particularly in severe cases like bloodstream infections.

Fosfomycin, a synthetic bactericidal antibiotic, is a phosphonic acid derivative (cis-1,2-epoxypropylphosphonic acid) that inhibits the first stage of peptidoglycan biosynthesis by binding to the MurA enzyme. It has a broad spectrum of antibacterial activity and a good safety profile, with low ecological impact on the intestinal microbiota. In vitro, it remains active against most ESBL-producing Enterobacteriaceae and some CPE isolates, particularly those carrying blaKPC-positive plasmids [2]. Amikacin, on the other hand, can disrupt the outer membrane of Gram-negative bacteria, potentially increasing the permeability of other antibacterial drugs [3,4,5]. This mechanism may enhance the penetration of antibiotics to their targets, leading to a synergistic bactericidal effect.

Although this antibiotic has its advantages, the risk of selecting for resistance mutations when used alone is high, as the frequency with which fosfomycin-resistant mutations are selected for in vitro is very high (from 10^{-7} to 10^{-6} cells). Therefore, fosfomycin is only recommended as monotherapy for uncomplicated urinary tract infections. In all other cases, it is recommended to use fosfomycin in combination with another active drug. Notably, several studies have reported that *E. coli* fosfomycin-resistant mutants appear to be less adaptable, explaining why clinical resistance has a broad spectrum of activity including ESBL-producing *E. coli*. However, the risk of developing drug resistance justifies its use as combination therapy rather than as monotherapy. However, based on our literature search, there are few data on the in vivo use of fosfomycin in combination with amikacin to treat CPE bloodstream infections. Therefore, we studied the efficacy of fosfomycin combined with amikacin in a mouse bloodstream infection model.

2. Materials and Methods

2.1. Patients and Strains

The strains in this study were derived from the blood culture of a child with bloodstream infection from the Children's Hospital Affiliated to Chongqing Medical University in a previous study on the clinical and microbiological characteristics of bloodstream infections with carbapenem-producing Enterobacteriaceae. in the blood sample in the bottle. Clinical data and microbial-related test results were obtained through the hospital information system, and a clinical strain of *Escherichia coli* was screened. Screening of heterogeneous drug resistance phenotypes was completed through in vitro experiments using the disk diffusion method and E-test strip method. It was found that the combination of amikacin and fosfomycin was effective against a carbapenemase-producing large intestinal *Escherichia coli* strain from a pediatric patient. Mycobacterium isolates had additive effects. VITEK MS (BioMerieux) was used to complete the identification of bacterial strains; the drug susceptibility

characteristics identified through VITEK fully automatic microbial identification and drug susceptibility system are: meropenem 8g/mL, imipenem 16g/mL, ertapenem 64g/mL, supplemented and verified drug susceptibility characteristics by disk diffusion method: imipenem 8g/mL, meropenem 16g/mL, ertapenem 64g/mL, amikacin 4g/mL. PCR and DNA sequencing technology were used to complete the identification of the carbapenemase encoding genes (blaKPC, blaNDM, blaIMP, blaVIM, blaOXA-48-like) of all strains; this strain carries the blaNDM-5 carbapenemase gene and blaTEM, ESBL genes such as blaCTXM use multilocus sequence analysis (MLST) method for molecular typing and were determined to be ST692 type.

2.2. Antibiotics

Antibiotics In all experiments, the antibiotics fosfomycin (macklin, Shanghai, China) and amikacin (macklin, Shanghai, China) were used. All in vitro experiments with fosfomycin were performed in media supplemented with 25 mg/L glucose-6-phosphate.

2.3. Disk Diffusion Methods

The double-disk diffusion method is to first perform single-disk diffusion of the bacteria. After culturing the strains on Columbia blood agar overnight, they are suspended in 0.9% physiological saline to reach 0.5 McFarland units (about 1.5×10^8 CFU/ml), then use a sterile cotton swab to spread the inoculum on the MH plate, and use tweezers to affix fosfomycin (including 25µg/ml G-6-P) drug-sensitive paper (200mg/L) and Amikacin drug susceptibility disk (30mg/L). The plates were then incubated at 37°C for 24 hours, and the inhibition zone diameters were read after 24 and 48 hours. Determine the diameter of each inhibition zone. Based on the single disk diffusion method, two drug-sensitive paper disks are flatly attached to the surface of MH agar medium. The sum of the distances between the centers of the drug-sensitive paper disks is half of the sum of the diameters of their respective inhibition zones. Incubate for 48h. Observe whether the heterogeneous drug-resistant colonies at the junction of the inhibitory zones disappear. If the colonies disappear, the combination is considered to have a potential synergistic effect.

2.4. E-Test

Overnight cultures on Columbia blood agar were suspended in 0.9% saline to achieve 0.5 McFarland units (1.5×10^8 CFU/mL), and then the inoculum was spread onto MH plates by sterile cotton swabs, and equivalent fosfomycin (including 25µg/ml G-

6-P) and amikacin MIC Test Strips (Liofilchem) were applied by tweezers. Plates were subsequently incubated for 24 h at 37°C, and MICs were read after 24 h and 48 h. Synergy tests were carried out according to the protocol of the MTSTM Synergy Applicator System of Liofilchem (25).

2.5. Agar Diffusion Checkerboard Test

Prepare a series of agar plates containing 25 mg/L G-6-P, with or without mixed antibiotics (fosfomycin and amikacin), arranged in an 8×8 matrix, FOS The concentration is from right to left, and the concentration of AK is from top to bottom, ranging from $1/16 \times \text{MIC}$ to $4 \times \text{MIC}$ (2-fold increments). The combination of

fosfomycin and amikacin was tested at concentrations of 0.125mg/L-8mg/L and 0.375mg-24mg/L. An overnight culture of 0.5 McFarland units was diluted 1:10 in 0.9% saline and 2 µl of the suspension was inoculated onto the plate. All plates were incubated at 37°C for 16-20 hours. New MICs for each isolate were determined for each drug alone or in combination. Calculate the fractional inhibitory concentration index (FICI) value according to the following formula $FICI = (MIC \text{ drug A with drug B} / MIC \text{ drug A alone}) + (MIC \text{ drug B with drug A} / MIC \text{ drug B alone})$. The FICI results were interpreted as synergistic (≤ 0.5), additive (> 0.5 to ≤ 1), indifferent (> 1 to < 4) or antagonism (≥ 4).

2.6. Experimental Bloodstream Infection

A total of 60 male C57BL/6J mice (Changsha, Hunan, China) weighing 20 ± 1 g and 6-8 weeks old were used. Each animal was injected with a bacterial inoculum of 1.5×10^9 CFU/ml into the tail vein, and the injection volume was 100µl. After 1 hour of observation. Animals were randomly assigned to four treatment groups. (i) FOS alone, (ii) AMK alone, (iii) both drugs in combination, and (iv) sterile PBS placebo. According to the group, the drugs were injected into the mice through the tail vein. Weigh and observe clinical manifestations.

2.7. Antibiotic treatment regimen

In order to make the antibiotics in plasma close to the standard intravenous injection regimen for humans, with reference to the concentrations of previous drugs administered in animal models, we conducted the effect concentration and safety of the concentrations of amikacin and fosfomycin administered to mice. Confirm the limits. Due to differences in drug efficacy/pharmacokinetics between animals and humans, there are differences in body weight between mice and humans, and between species. Taking fosfomycin as an example, the human clinical dosage is 25 mg/mouse based on the drug conversion formula for humans and mice. The median lethal dose of fosfomycin in mice was found to be 1225 mg/kg. It is easy to cause acute drug poisoning and death in mice. In fact, during our experiment, when this concentration of fosfomycin was injected into model mice, the mortality rate was 100%. Reduce 4mg/mouse, that is, 200mg/kg. The concentration of amikacin is 20mg/kg. According to experiments, it is safe for mice at this concentration.

2.8. Bacterial Counting

24 hours after completing antibacterial treatment, the mice were euthanized with sodium pentobarbital, blood was taken from the heart cavity, and the liver and spleen of the mice were taken. Add 1 ml of PBS to each specimen, transfer it to a glass tissue homogenizer and grind it into a suspension. Prepare a 10-fold dilution series pipette 5ul of the dilution and inoculate it onto a Columbia blood agar plate and repeat three times. After incubation at 37°C for 24 hours, count the colonies and express the results as CFU/5ul or log₁₀ CFU/mL.

2.9. Histopathology

For histological confirmation of bloodstream infection, livers and spleens from mice in each group were classically embedded, and thin sections were stained with hematoxylin and Y in red.

2.10. Statistics

Experimental results were plotted and analyzed using GraphPad Prism, version 8.0 (GraphPad Software Inc.) and SPSS 22.0 for Windows NT software (SPSS Inc.), and analyzed using the Mann-Whitney U test, followed by the Bonferroni multiple comparison test. P value <0.05 is considered statistically significant.

3. Results and Discussion

3.1. Construction of bloodstream infection model

After constructing the bloodstream infection model, we found that few target strains were isolated from the blood of mice. The reason is that blood circulation and immune clearance make the concentration of bacteria in living mice lower than that in other organs, making it difficult to obtain them through culture. In the same way, bloodstream infections in clinical patients also need to be increased through culture bottles to increase the positive rate. According to literature reports, bacteria reach various organs, such as liver, spleen, kidneys, and lungs, at different times after bloodstream infection. The peak and subsidence times are also different in these organs. Within 24 hours, the bacterial load of the organs was much higher than that between 48 and 72 hours. The liver was larger than the spleen, the kidneys were larger than the lungs. Among the four observation organs we selected: liver, spleen, kidney, and lung, the liver and spleen recovered the largest amount of bacteria. The bacterial loads in the lungs and kidneys were lower. (Figure 2A) Within 72 hours after intravenous injection of bacterial suspension into the tail vein, the weight of mice decreased fastest in the first 24 hours (Figure 2B).

3.2. Antibacterial activity of the combination in vivo experiments

5 out of 61 mice died during anesthesia and were not analyzed further. After bloodstream infection with clinical strains of *E. coli*, 5×10^8 CFU 24 hours after bacterial infection, under different antibiotic regimens, Figure 2C shows the quantitative results of the antibiotic treatment regimen for experimental bloodstream infections, as organ grinding fluid log₁₀ CFU/ml express. The bacterial count (mean $\pm$ standard deviation) of the control group (n = 9) was 7.17 ± 0.48 log₁₀ CFU/mL. FOS treatment (n=13) resulted in a bacterial count of 6.72 ± 0.24 log₁₀ CFU/mL, which was significantly better than the control group (P<0.05). The bacterial count of FOS and AMK combined treatment (n=12) was 6.74 ± 0.30 log₁₀ CFU/mL, which was significantly better than that of the control group (P<0.05). AMK treatment (n=13) resulted in a bacterial count of 6.98 ± 0.32 log₁₀ CFU/ml, which was not significantly different.

Therefore, the results of combined treatment of FOS at a dose of 200 mg/kg body weight and AMK at a daily dose of 60 mg/kg body weight were significantly better than those of the placebo group and AMK single drug group (P<0.05). The combined use of FOS and AMK was not more effective than FOS alone (P=1.0)

Histopathological evaluation: The histological specimens of one mouse randomly selected from each of the four groups showed that the liver and spleen of the mice were stained with HE and the liver (Figure 3A) and spleen (Figure 3B) specimens were fixed and sectioned for analysis. HE staining. The liver of the drug-free regimen showed infiltration of inflammatory cells (blue arrow), in which the liver tissue showed inflammatory damage, fatty degeneration and necrosis, and a large number of vacuoles appeared in the liver cells (red arrow). Infiltration of inflammatory cells in the spleen. However, there was no significant difference in the scope of inflammatory

response and degree of damage between different medication regimens. There were no significant differences in serum liver function (ALP,AST).

Antibiotic resistance: All *Escherichia coli* strains recovered at 24 hours were tested for antibiotic susceptibility to FOS and AMK. The MICs were all within 1 dilution of the MIC before treatment, indicating that there was no increase in drug resistance in either monotherapy or combination therapy.

Discussion: Phenoxymethylpenicillin, in combination with other drugs, has been proven to enhance the antibacterial effect of various antibiotics against CPE Gram-negative bacilli. Stéphane Corvec reported on the in vitro and in vivo activity of phenoxymethylpenicillin, tigecycline, polymyxin, and gentamicin (alone and in combination) against a well-characterized ESBL-producing clinical strain of *Escherichia coli* in an implant infection model [6]. The in vitro time-kill curves did not show combined antibacterial activity, but in the animal model study of foreign body infection, phenoxymethylpenicillin alone could reduce the planktonic colony count by 4 log₁₀ CFU/mL, while in combination with polymyxin, tigecycline, or gentamicin, it could reduce more than 6 log₁₀ CFU/mL.

The differences between in vitro results and observations from animal studies are not surprising, as the concentration of antibiotics in target organs during severe infections such as bloodstream infections and sepsis is difficult to determine and may differ greatly from serum levels. Moreover, in vitro results cannot reflect the additional immunomodulatory properties of antibiotics. Mouse models of bloodstream infections currently mainly include cecal ligation and puncture bacteremia models and direct bloodstream infection models. In addition, there are local organ-related infection models. They have all been reported for evaluating the therapeutic effects of antibiotics. Using the Cecal Ligation and Puncture (CLP) method [7], which is a common surgical technique that induces peritoneal infection and subsequent bacteremia by ligating and puncturing the cecum, mouse models of bacteremia are widely used to study disease mechanisms related to bacteremia, drug efficacy, and the impact on disease prognosis. This model can be used to study infection, inflammation, immune response, etc., helping to reveal the pathogenesis and influencing factors of bacteremia. Although mouse models of bacteremia have many advantages, such as simulating the human disease process and low cost, they also have some limitations. For example, due to the diversity of phenotypes and inter-individual variability in human bacteremia, it is difficult to develop and control, leading to mouse death before the experiment is completed. A comprehensive animal model remains very difficult. Direct bloodstream infection can study the bloodstream infection of a single strain in the construction model. However, it also exists that it is quickly cleared by the immune system in mice, and it may not be able to fully simulate the process of antibiotics exerting antibacterial activity in the human body. It also cannot fully and accurately simulate the survival situation of severe bloodstream infections in humans. Literature reports that invertebrates such as the *Galleria mellonella* can be used as research models for survival situations under infection states [8].

In this study, the bloodstream infection model was used to evaluate the therapeutic efficacy of FOS and AMK combined in bone tissue. We found that for this clinically isolated strain of *Escherichia coli*, it showed heterogeneous resistance to both FOS and AMK when tested individually in vitro. However, when combined in culture medium, they exhibited additive antibacterial activity. Therefore, we further reproduced this antibacterial activity through an animal model.

For human intravenous injection protocols, FOS is given at 4-12g per day for adults, and can be increased to 16g per day for severe infections, administered 2-3 times a day. For amikacin, the clinical intravenous treatment regimen is: 7.5mg/kg every 12 hours for systemic infections, or 15mg/kg every 24 hours. We did not find a literature report on the dosage of fosfomycin for direct tail vein injection in mice. There is a significant difference in tolerance to the same drug between humans and animals; generally, animals have a higher tolerance than humans. We used the conversion formula between the body surface area of animals and humans to calculate that for mice weighing 20g, the dosage of FOS would be 400-1250mg/kg, and AMK would be 20mg/kg. As reported, the median lethal dose (LD50) of FOS in mice is 1225mg/kg, and the LD50 of AMK is 181mg/kg. In the actual experiment, after injecting mice with FOS at this concentration, the mice showed acute toxic reactions and all died within 1 minute after injection, confirming that this concentration of FOS is lethal to mice. Amikacin, however, showed no toxic reaction. After reducing the concentration of FOS and administering it, we determined a safer dosage concentration of FOS to be 200mg/kg once every 24 hours, or 100mg/kg twice every 24 hours. At the same time, we verified through mice that acute toxic reactions did not occur at this concentration.

Our research also explained that after tail vein injection of bacterial suspension, the bacterial load in various organs of the surviving mice varied greatly. The possible reasons for this are not only related to the differences in bacterial colonization caused by the blood circulation of various organs, which leads to different half-lives of bacteria in different organs. At the same time, it is undeniable that different bacterial species have different preferences for colonization in different organs. For example, *Klebsiella pneumoniae* can lead to liver abscesses. In our study, we found that in the absence of any drug intervention, only after injecting the bacterial suspension, the bacteria in the bloodstream of infected mice would be cleared from various organs and peripheral blood circulation over time. Especially after 48-72 hours of infection, the bacterial load in almost all organs decreased. After 72 hours, the target strain could not be cultured. Therefore, 24 hours is a suitable time for us to observe the antibacterial activity of drugs in mice. At the same time, since the bacterial content in blood is extremely low without enrichment culture, our research confirmed that the liver and spleen are good target organs for detecting changes in bloodstream infection bacteria. However, at the same time, not only the colonization of bacteria in various organs of mice is different, but also the concentration of antibiotics accumulated in the tissues of humans and other mammals such as mice, and the time of subsidence are also different. This also confirms the significance of comparative research between in vivo and in vitro antibiotic experiments. The value of in vivo experiments lies in the stable and constant bacterial and drug concentrations of in vitro experiments. Surprisingly, in this study, fosfomycin monotherapy showed heterogeneous resistance to *E.coli* strains in vitro, but in mice, even at a low-dose regimen, it showed antibacterial activity compared to the non-treatment group. FOS treatment was so effective that when used in combination with AMK, no synergistic effect was observed. It is noteworthy that no antagonistic effect was observed. Different from the microbiological evaluation results in our study, although there were differences in antibacterial activity among different treatment groups in the liver, there was no significant difference in the severity of inflammatory damage histopathologically. This is not surprising, because histopathology reflects that the process of tissue and organ inflammatory response may lag behind bacterial clearance in terms of duration and degree of change. In our study, no enhanced resistance was

detected in the samples of *Escherichia coli* recovered after treatment, that is, the MIC did not increase significantly. This supports the results of W.Poeppel [9] and others' study on MRSA osteomyelitis in rats with FOS and daptomycin for 4 weeks. However, Thauvin [10] et al. found that when FOS was used as a single drug at a dose of 500mg/kg body weight per day for 6 days in experimental rat endocarditis, the development rate of resistance was 36%. When FOS was used in combination with pefloxacin, no resistance developed. Of course, the length of the treatment cycle, the drug concentration in the target organ, and the differences in model animals of different species may all lead to the occurrence or non-occurrence of bacterial resistance in vivo. We need more validation in mouse infection models through different concentrations, drug action times, and different target organs to see if bacterial resistance occurs due to antibiotic regimens. It should be noted that there are limitations to the study. First, all experiments were conducted using the same single clinical isolate of primary CPE bacteria preserved. Second, bloodstream infection is a heterogeneous disease with a variety of disease manifestations and pathophysiology, and there is no single model that encompasses all aspects of bloodstream infection. At the same time, this strain originated from pediatric patients with bacteremia, and there are differences in immune function between children and adults. There are also differences in the clinical manifestations of inflammatory responses. Therefore, it is not yet known whether the data obtained from this study can be extrapolated to other models of anatomical sites such as urinary tract infections and intra-abdominal infections.

4. Conclusions

This study shows that the heterogeneous resistance phenotype of *Escherichia coli* strains that exhibit heterogeneous resistance to FOS and AMK disappears after combined treatment with FOS and AMK. At the same time, in animal infection models, FOS showed good antibacterial effects when used alone, but did not show a synergistic effect when used in combination with AMK. In summary, AMK in vitro can synergistically enhance the antibacterial effect of FOS, causing heterogeneous drug-resistant *Escherichia coli* to become sensitive again. In vivo studies have found no synergistic effect between AMK and FOS.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

This work was supported by the National Natural Science Foundation of China (Approval No. 82002255), Chongqing Natural Science Foundation (Approval Nos. CSTB2023NSCQ-MSX0179, cstc2021jcyj-msxmX0217, and cstc2020jcyj-msxmX0519), Chongqing Medical Science Research Project (Joint project of Chongqing Health Commission and Science and Technology Bureau) (Approval No. 2022QNXM002), Chongqing Medical University's Future Medicine Youth Innovation Plan, and the 2020 Chongqing Graduate Research and Innovation Project (Approval No. CYS20191).

Ethical Approval and Participant Consent

The data and isolates analyzed in this study were obtained according to standard procedures and were approved by the Institutional Medical Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University (Ethics Review Approval Number: K2023-293). The isolates and clinical samples were collected in the microbiology laboratory of the hospital without patient contact. Therefore, the Institutional Medical Ethics Review Committee determined that informed consent was not required.

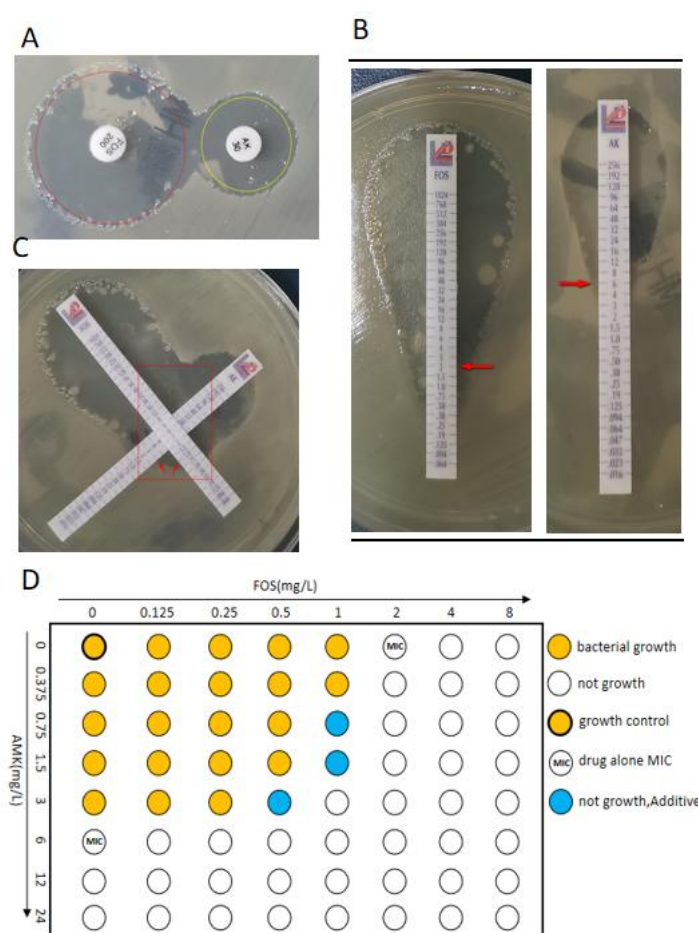


Figure 1. The combination of FOS and AMK showed an additive effect on strain. (A) Dual K-B tests. The results showed that the effect of the combination of FOS and AMK on the strain was additive. colony at the interface of the two antibiotic disks. (B-C) The modified E-test showed that the combination of FOS and AK on strain was additive (FICI = 1) according to the interpretation by Liofilchem. Red arrows on the pictures point to MICs. The E-test results of strain to AMK and FOS. Red rectangle: there was no colony at the interface of the two antibiotic strips. (D) A schematic of the checkerboard assay: the FICI of the assay was 0.75, indicating additive effect.

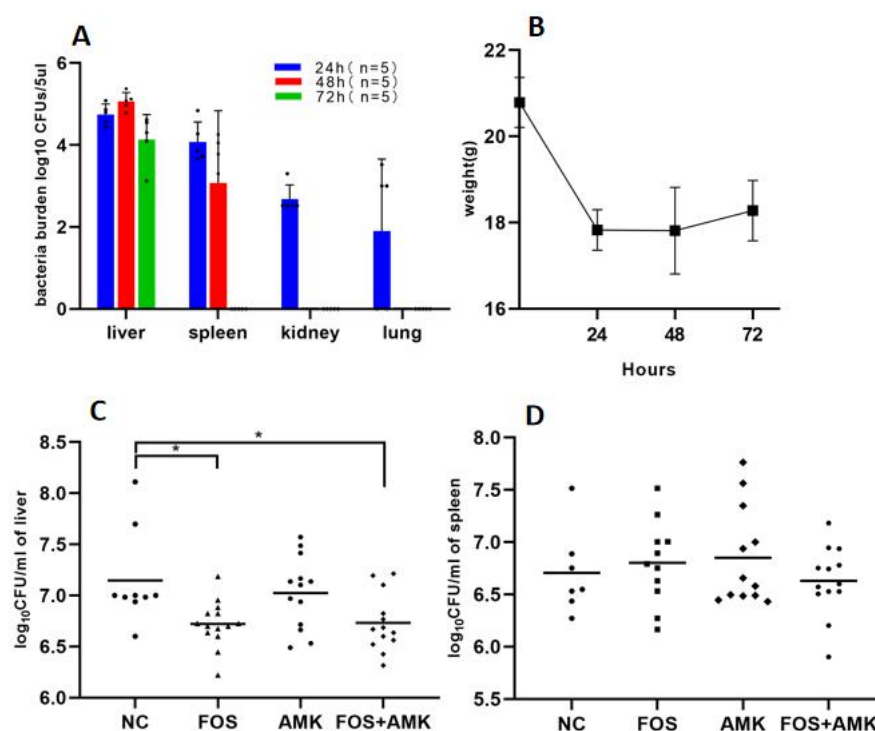


Figure 2. Results of the experimental bloodstream infection mouse model. (A) Bacterial load in different organs of mice after infection, at 24 hours, 48 hours, and 72 hours without treatment (B) Weight changes of mice after infection under untreated conditions (C) Bacterial load in the liver of mice under different treatment regimens (D) Bacterial load in the spleen of mice under different treatment regimens.

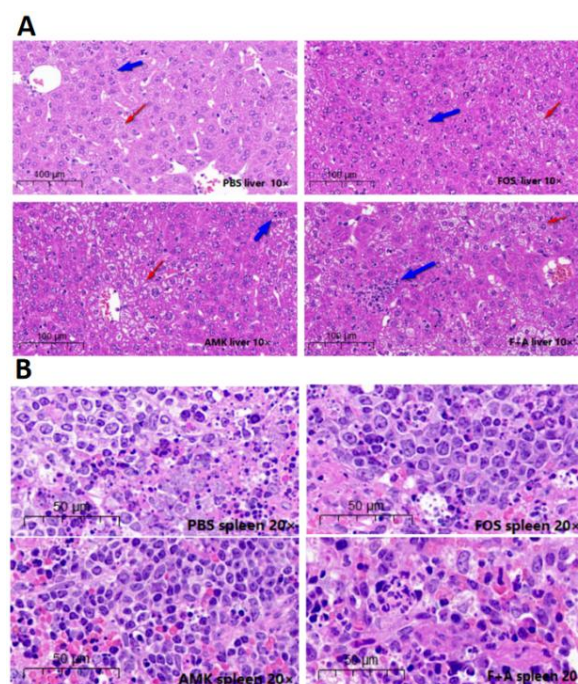


Figure 3. Histological characteristics. Pathological pictures of liver (A) (Giemsa stain) (original magnification, $\times 10$). and spleen (B) (Giemsa stain) (original magnification, $\times 20$). under different antibiotic dosing regimens 24 hours after 1.5×10^8 CFU bacterial infection. Inflammatory cell infiltration was demonstrated in liver and spleen under different dosing regimens, in which the liver tissue showed inflammatory injury steatosis-like degenerative necrosis. However, there was no significant difference in the extent of inflammatory reaction and damage between different dosing regimens.

References

- [1] Nordmann, P.; Naas, T.; Poirel, L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerging infectious diseases*, 2011, 17(10), 1791-8.
- [2] Falagas, M.E.; Giannopoulou, K.P.; Kokolakis, G.N. et al. Fosfomycin: use beyond urinary tract and gastrointestinal infections. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 2008, 46(7), 1069-77.
- [3] Mizgerd, J.P.; Skerrett, S.J. Animal models of human pneumonia. *American journal of physiology Lung cellular and molecular physiology*, 2008, 294(3), L387-98.
- [4] Bulitta, J.B.; Ly, N.S.; Landersdorfer, C.B, et al. Two mechanisms of killing of *Pseudomonas aeruginosa* by tobramycin assessed at multiple inocula via mechanism-based modeling. *Antimicrobial agents and chemotherapy*, 2015, 59(4), 2315-27.
- [5] Yadav, R.; Landersdorfer, C.B.; Nation, R.L. et al. Novel approach to optimize synergistic carbapenem-aminoglycoside combinations against carbapenem-resistant *Acinetobacter baumannii*. *Antimicrobial agents and chemotherapy*, 2015, 59(4), 2286-98.
- [6] Corvec, S.; Furustrand, T.; Ulfarsson, U.; Betrisey, B. et al. Activities of fosfomycin, tigecycline, colistin, and gentamicin against extended-spectrum- β -lactamase-producing *Escherichia coli* in a foreign-body infection model. *Antimicrobial agents and chemotherapy*, 2013, 57(3), 1421-7.
- [7] Drechsler S.; Osuchowski M. Cecal Ligation and Puncture. *Methods in molecular biology (Clifton, NJ)*, 2021, 2321, 1-8.
- [8] Pereira, M.F.; Rossi, C.C.; da Silva, G.C. et al. *Galleria mellonella* as an infection model: an in-depth look at why it works and practical considerations for successful application. *Pathogens and disease*, 2020, 78(8).
- [9] Poepl, W.; Tobudic, S.; Lingscheid, T. et al. Daptomycin, fosfomycin, or both for treatment of methicillin-resistant *Staphylococcus aureus* osteomyelitis in an experimental rat model. *Antimicrobial agents and chemotherapy*, 2011, 55(11), 4999-5003.
- [10] Thauvin, C.; Lemeland, J.F.; Humbert, G. et al. Efficacy of pefloxacin-fosfomycin in experimental endocarditis caused by methicillin-resistant *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy*, 1988, 32(6), 919-21.



© 2024 by the author(s); licensee International Technology and Science Publications (ITS), this work for open access publication is under the Creative Commons Attribution International License (CC BY 4.0). (<http://creativecommons.org/licenses/by/4.0/>)