Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.120

RESEARCH ARTICLE

In vitro Evaluation of Antibiotic–Matrine Combinations Against Clinically Relevant Bacteria

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ARTICLE HISTORY (23-397)

Received: September 9, 2023
Revised: December 5, 2023
Accepted: December 7, 2023
Published online: December 28, 2023

Key words:
Antibiotic
Matrine
Minimal Inhibitory Concentration (MIC)
Mutant Prevention Concentration (MPC)
Mutant Selection Window (MSW).

ABSTRACT

Antibiotics are widely used as the most effective treatment for bacterial infections. However, the extensive use of antibiotics in clinical practice has led to elevated levels of bacterial antibiotic resistance in common clinical pathogens. One potential solution to this problem is the use of traditional Chinese herbal medicines to supplement antibiotic effectiveness. In this study, we utilized the matrine derived from the leguminous shrub *Sophora flavescens* and examined synergism with 17 clinically relevant antibiotics against *Staphylococcus aureus*, β-hemolytic *Streptococcus, Pasteurella multocida*, *Escherichia coli* and *Salmonella paratyphi* B using *in vitro* tests. Matrine exhibited significant antibacterial activity against these bacteria with minimum inhibitory concentrations (MIC) ranging from 4.69 to 9.38 mg/mL. Matrine combinations also yielded fractional inhibitory index values between 0.14 and 1 indicating additive or synergistic effects without antagonism. Furthermore, the mutation prevention concentration (MPC) analysis revealed that matrine could mitigate the impact of ceftiofur, doxycycline, gentamicin and tilmicosin for all 5 bacterial strains. The reduced MPC and MPC/MIC values of these antibiotics demonstrated a narrower selection window for drug-resistance mutations thereby retarding the development of drug resistance. The combination of matrine and antibiotics thereby enhanced the antibacterial activity of all test antibiotics while reducing the antibacterial impact of ceftiofur, doxycycline, gentamicin and tilmicosin on *S. aureus* and β-hemolytic *Streptococcus*. Additionally, matrine combinations with the latter drugs could lower the anti-mutation concentration thereby reducing the emergence of drug resistant strains.

To Cite This Article: Wu H, Tao M, Liu J, Zhang X, Hu J, Ma B and Chen P, 2024. *In vitro* evaluation of antibiotic – matrine combinations against clinically relevant bacteria. Pak Vet J, 44(1): 162-168. http://dx.doi.org/10.29261/pakvetj/2023.120

INTRODUCTION

Bacterial drug resistance is an ongoing significant global concern. The misuse and overuse of antibiotics in animal husbandry and disease treatment have resulted in the emergence and widespread dissemination of drug-resistant bacteria and consequently, multi-drug resistance (MDR) and cross-resistance in bacterial pathogens (Rather *et al*., 2021; Upadhayay *et al*., 2023). This has imposed challenges for treatment of bacterial infections particularly in livestock and poultry diseases. Furthermore, the use of untreated livestock and poultry manure as fertilizer introduces drug-resistant bacteria and their genes into soils facilitating the spread of drug resistance (Larsson and Flach, 2022). Antibiotics from animal-derived food as well as veterinary drugs in human environments also poses a threat to human health (Hussain *et al*., 2021; Mangla *et al*., 2022). Drug-resistant bacteria also present significant clinical challenges and impose a substantial economic burden on society. For instance, the United States has experienced an annual increase of 3.8% ($1.2 trillion) in financial burdens for the control of MDR bacterial infections (Alkufeidy, 2022). Consequently, scientists are actively exploring new types of antibiotics and innovative strategies to combat the problem of drug resistance to address this formidable threat.

Numerous studies have demonstrated the extensive potential of Chinese herbal medicine in clinical applications due to its long-standing history, abundant

resources, potent antibacterial effects and diverse pharmacological properties (Cyranoski, 2018). Consequently, the identification of effective antibacterial drugs or drug resistance inhibitors from traditional Chinese herbal medicines has emerged as a viable strategy to address the issue of bacterial drug resistance. Matrine (CAS 519-02-8) is an alkaloid present in leguminous shrubs of the genus *Sophora* (Fabaceae) such as *Sophora flavescens* and Chinese herbal medicinal preparations are known as Dogel ebs. Matrine exhibits a broad spectrum of bactericidal effects and a limited propensity to induce drug resistance. The compound possesses low toxicity for animals and plants and as such, presents negligible or minimal residual effects to the environment. Matrine holds great promise for the treatment of numerous clinical conditions (Li *et al*., 2021). Matrine has demonstrated significant pharmacological activity against porcine reproductive and respiratory syndrome virus (Zhao *et al*., 2023), *Staphylococcus aureus* (Zhao *et al*., 2017), *Escherichia coli* (Huang, 2017) and *Salmonella enteritidis* (Li, 2023).

The current study was conducted to investigate the antibacterial activity, antimutagenic properties and resistance reduction of matrine in combination with 17 typical antibiotics used for clinical treatment of animal infections. This work lays a foundation for exploring the potential of matrine to control the spread of drug-resistant bacteria through empirical data and theoretical evidence.

MATERIALS AND METHODS

Bacterial test strains: The standard strains used in this study were obtained from Beijing Preserved Biotechnology (Batch 20230227) and included *E. coli* (CMCC 44102), *S. aureus* (CMCC 26003), *Pasteurella multocida* (CVCC 393), *Salmonella paratyphi* B (CMCC 50094) and β-hemolytic *Streptococcus* (CMCC 32210) or BHS.

Antibacterial drugs: Matrine purchased (≥ 98%) was obtained from Shaanxi Xintianyu Biotechnology (Shaanxim, China). Penicillin sodium (≥ 95.2%, Cat. No. YZ202302473), ampicillin sodium (≥ 95%, Cat. No. YZ202302261), amoxicillin sodium (≥98%, Cat. No. YZ202301392), ceftiofur sodium (purity ≥95%, Cat. No. YZ202302727) and cefquinome (≥ 87.3%, Cat. No. YZ202301392) were purchased from Hebei Yuanzheng Pharmaceutical (Hebei, China). Gentamycin sulfate (≥94%, Cat. No. HB202211051), kanamycin sulfate (≥94.3%, Cat. No. HB202212026), amikacin sulfate (≥95%, Cat. No. HB202211037), neomycin sulfate (≥93 %, Cat. No. HB202301036) and streptomycin sulfate (≥ 94.2% Cat. No. HB202301047) were purchased from North China Pharmaceutical Group (Shijiazhuang, China) Doxycycline hydrochloride (≥96.2%, Cat. No. RP202302364), aureomycin hydrochloride (≥97%, Cat. No. RP202302362), oxytetracycline hydrochloride (≥97%, Cat. No. RP202302363), erythromycin (≥98%, Cat. No. WH202207542), tylosin tartrate (≥98%, Cat. No. WH202207544), tilmicosin (≥98%, Cat. No. WH202207543) and tyramycin (≥98%, Cat. No. WH202207545) were purchased from Tianjin Ringpu Biotechnology (Tianjin, China).

Determination of the minimum inhibitory concentration of single drugs: Antimicrobial testing was conducted using the standard microdilution method recommended by the National Committee for Standardization of Clinical Laboratories (NCCLS, 2022). In brief, minimal inhibitory concentration (MIC) values were determined using single isolated plate colonies cultured in broth medium (see below) at 37°C for 12-18 h. The concentration of the bacterial solution was adjusted to 0.5 McFarland units (1×10^8 colony-forming units (CFU)/mL) and diluted with broth medium to 1×10^6 CFU/mL. Serial dilutions of the test drug were added (100 µL) to each row of a 96-well microwell plate in sequence followed by addition of 100 µL diluted bacteria. Positive controls contained only bacteria diluted with 100 µL broth and the negative control contained only broth. The plate was incubated at 37°C for 16-18 h. The MICs were recorded as the drug dilution corresponding to the first well in a series lacking bacterial growth. Luria Bertani broth was used for *E. coli* tests, nutrient broth for *Salmonella*, *Staphylococcus* and *Pasteurella* and BHS required glucose meat infusion broth.

Determination of Minimal Inhibitory Concentration of Combined Drugs: Combination drug experiments were conducted using the micro checkerboard dilution method (Mei *et al.*, 2019) as per above for MIC determinations using 2-fold serial antibiotic dilutions beginning with 2 × MIC and a constant level of matrine in each well. Distilled water was used for matrine dissolution and for matrine blank wells and used at 50 µL per well added to 100 µL volumes of bacterial solution and antibiotic, respectively. Appropriate positive, negative and matrine single drug controls were included with each plate group. Plates were incubated at 37°C for 24 h and scored for MIC level as per above. The fractional inhibitory concentration index (FICI) was calculated using the following formula and used to evaluate the combined drug effects:

$$\sum FICI = C_A/MIC_A + C_B/MIC_B$$

Where MIC_A and MIC_B represent the MICs of drugs when used individually and C_A and C_B represent the concentrations of the two drugs when they are used in combination to achieve the same effect. The FICI was defined as follows: FICI ≤0.5, synergy; 0.5 <FICI ≤1, additivity; 1< FICI ≤2, indifference; FICI> 2, antagonism.

Determination of single-drug MPCpr and MPC: The minimum preventive concentration (MPC) for the drugs utilized in these tests was determined using bacterial solutions prepared as per above. Single plate colonies were inoculated in 5 mL nutrient broth and incubated overnight at 37°C with shaking at 2500 RPM. The cultures were centrifuged and cell pellets were suspended in 50 mL nutrient broth and incubated 6 h to reach 1×10^9 CFU/mL. The bacterial cells were again pelleted by centrifugation and the concentration was adjusted to 3×10^{10} CFU/mL.

The MPC of each drug was determined as previously described (Pasquali and Manfreda 2007). In brief, drug dilutions were used at 64, 32, 16, 8, 4 and 2 × MIC incorporated into agar plates. The plates were then coated with 100 µL of 3×10^{10} CFU/mL bacteria and incubated at 37°C for 72 h. The lowest drug concentration at which no

bacterial growth was observed was considered as the provisional mutation prevention concentration (MPCpr). This concentration was then gradually decreased by 20% to 1/2 MPCpr to prepare agar plates. The same amounts of bacteria were inoculated on these plates and after 72 h the lowest concentration without bacterial growth was determined as the accurate MPC. Therefore, the minimum selective window (MSW) of the drug against the bacteria was defined as the range between MIC and MPC.

Determination of MPCpr and MPC after combined administration of antibiotics and matrine: The agar plate checkerboard dilution method (see above) was again utilized to determine the MPC of matrine and antibiotic combinations. The concentration was initially set at 0.5 × MIC for a single drug and subsequently multiplied until the MPC of the drug was reached as per above. Employing a ratio of 1 mL matrine, 1 mL antibiotic and 18 mL nutrient agar, plates containing both drugs were prepared using cross-combinations in triplicate. The minimum synergistic width or mutation selection window (MSW) of matrine combined with antibiotics was represented by the ratio of the combined MIC and combined MPC.

RESULTS

MIC determinations and combined drug susceptibilities: We initially determined the MIC values for all test antibiotics (17) and matrine against *S. aureus*, BHS *Streptococcus*, *P. multocida*, *E. coli* and *S. paratyphi* B. Specifically, the MIC range for the antibiotics and matrine against *E. coli* were 0.62-781 μg/mL and 9.375-18.75 mg/mL, respectively. In contrast, combined antibiotic-matrine MIC values fell in the range of 0.122 -98.000 μg/mL. Additionally, the MIC range of the matrine was 0.146-9.375 mg/mL with an FICI range of 0.140 - 1.030 (Table 1). These findings indicated that matrine and antibiotic combinations exhibited a synergistic or additive effect against *E. coli*. Notably, the aminoglycosides streptomycin, neomycin, gentamicin and kanamycin as well as the macrolides tylosin, tilmicosin and tyramycin demonstrated synergy with matrine i.e., FICI 0.140 - 0.380.

Tests using BHS resulted in MIC values ranging from 3.9 - 391 μg/mL while the MIC for matrine was 9.375 mg/mL. The MIC range of antibiotic-matrine combinations were 0.244 - 98 μg/mL and 0.293 - 4.687 mg/mL for matrine. The FICI ranged from 0.187 - 1 indicating synergistic or additive effects. Specifically, the macrolides tylosin, tilmicosin and tyramycin combined with matrine generated synergism with a FICI range of 0.187-0.375 (Table 2).

The MIC range for our test antibiotics and matrine against *S. paratyphi* B were 3.906 -391 μg/mL and 9.375 mg/mL, respectively. When used together, the antibiotic MIC range decreased to 0.625-39 μg/mL and the MIC range for matrine decreased to 0.293-4.688 mg/mL. The combination of antibiotics and matrine exhibited FICI values ranging from 0.250-1.031 indicating mostly synergistic or additive effects (Table 3).

The assay results for *P. multocida* generated antibiotic and matrine MIC ranges of 0.620-391 μg/mL and 2.344-18.750 mg/mL, respectively. Antibiotic-matrine combinations exhibited an MIC range of 0.122-78.1 μg/mL for the antibiotics and 0.049-9.375 mg/mL for matrine. The FICI values fell withing the range 0.187-1.031 that represented a predominantly synergistic or additive effect (Table 4).

The MIC range for our test antibiotics and matrine against *S. aureus* were 0.244-39.100 μg/mL and 4.688-9.375 mg/mL, respectively. When used together, the MIC range for antibiotics were 0.120-6 μg/mL and for matrine were 0.293-4.688 mg/mL. The FICI range was 0.312 -1 indicating a synergistic or additive effect (Table 5).

Matrine lowers the mutant selection window (MSW) of test antibiotics: Matrine combined with our test antibiotics also displayed anti-mutation effects. Specifically, the mutation selection window (MSW) of ceftiofur against *E. coli*, *S. aureus*, *S. paratyphi* B, *P. multocida* and BHS were 0.630-5.040, 1.250-5, 12.5-100, 0.625-10 and 32-256 μg/mL, respectively. When combined with matrine, these MSW values decreased to 0.160-0.640, 0.625-2.5, 1.56-10, 0.160-2.5 and 16-128 μg/mL for the respective microorganisms. However, the selective index (SI) value dropped to 1-2 times the original value (Fig. 1A).

The MSW of doxycycline against *E. coli*, *S. aureus*, *S. paratyphi*, *P. multocida*, and BHS were 3.906 -62.5, 0.031-2, 39.062-625, 3.906-62.5 and 78.125-640 μg/mL, respectively. The SI values ranged from 16 to 64. When combined with matrine, the MSW of doxycycline decreased to 1.953 - 15.625, 0.015-0.122, 19.531-156.25, 0.976-15.625 and 19.531-160 μg/mL, respectively. The SI values dropped 8-16 times the original value (Fig. 1B).

Gentamicin displayed MSW values against *E. coli*, *S, aureus*, *S. paratyphi*, *P. multocida* and BHS of 0.388-24.83, 0.156-5, 3.906-31.25, 0.977-62.5 and 3.906-62.5 μg/mL, respectively. The gentamicin- matrine combinations displayed MSW values of 0.122-3.904, 0.190-0.760, 0.977-15.625, 0.122-7.808 and 1.954-15.625 μg/mL, respectively. The SI value decreased from 8-64 to 4-64 after the combined use of gentamicin and matrine (Fig. 1C).

The MICs for tilmicosin against *E. coli*, *S, aureus*, *S. paratyphi*, *P. multocida* and BHS ranged from 391 to 3128 μg/mL and 12.2-97.6 μg/mL. When combined with matrine, these MICs were lowered to 98-391, 1.5-12 μg/mL, 19.5-156, 12.18-48.75 and 49-195 μg/mL, respectively. The SI values that indicated the therapeutic index, was initially 4-8, but it dropped to 1-2 times the original value after combined with matrine (Fig 1D).

DISCUSSION

The increasing use of antimicrobial drugs has led to elevated levels of antibiotic resistance in clinical bacterial infections. MDR bacteria pose a global threat and it is crucial that effective treatments for MDR infections are found (Hughes and Andersson 2017). Traditional Chinese medicines are known for its multi-component, multi-target and multi-action approach and these drugs have shown no significant bacterial drug resistance in long-term clinical applications (Ma *et al*., 2018). For instance, Chinese medicines possess antibacterial properties but can also

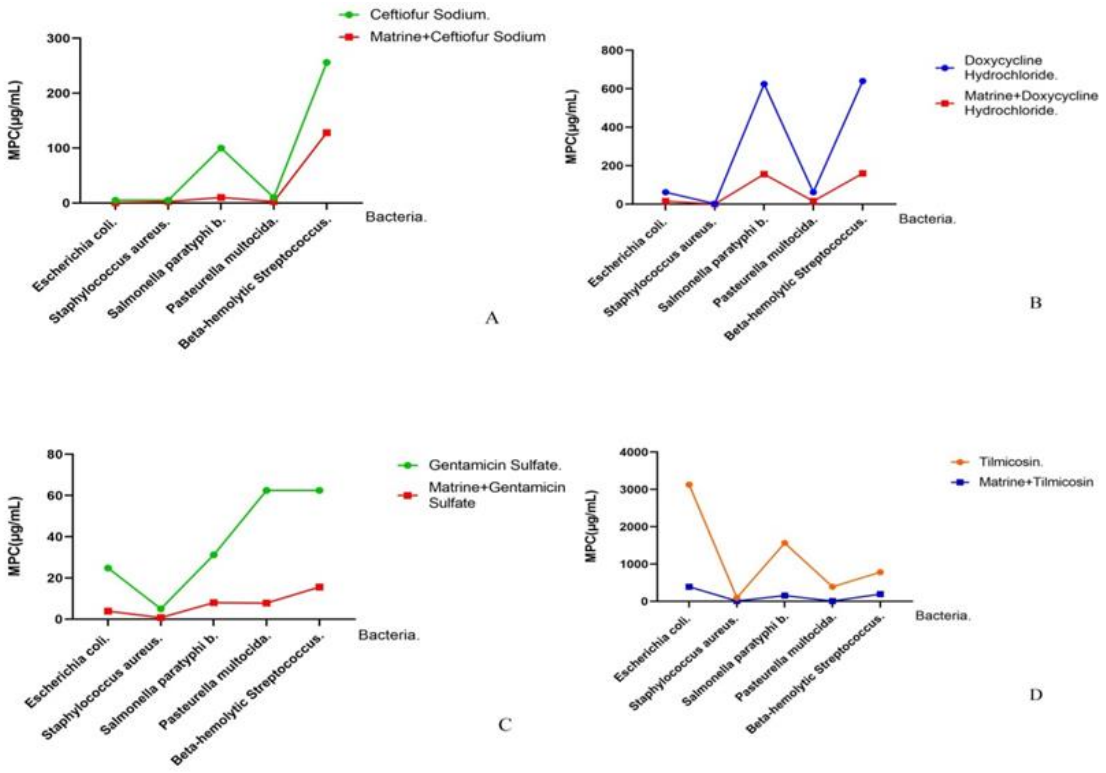

Fig. 1: The MPC for antibiotic alone and in combination with matrine against *Escherichia coli,Staphylococcus aureus,Salmonella paratyphi b, Pasteurella multocida, Beta-hemolytic Streptococcus*.: (A) ceftiofur or ceftiofur and matrine .(B) doxycycline or doxycycline and matrine.(C) gentamicin or gentamicin and matrine.(D) tilmicosin or tilmicosin and matrine.

Table 1: Combined Drug Sensitivity of Some Antibiotics and Matrine to *E. coli.*

Drug	Antibiotics（μg/mL）		Matrine（mg/mL）		FICI	Results
	MIC_A	C_A	MIC_B	C_B		
Ceftiofur Sodium	0.630	0.160	9.375	2.343	0.50	synergy
Streptomycin sulfate	1.953	0.244	9.375	2.344	0.38	synergy
Neomycin sulfate	3.906	0.61	18.75	4.687	0.41	synergy
Gentamicin Sulfate	0.977	0.122	9.375	0.146	0.14	synergy
Kanamycin Sulfate	0.977	0.122	9.375	1.172	0.25	synergy
Tylosin tartrate	312.500	78.100	9.375	1.172	0.38	synergy
Tilmicosin	781.000	98.000	9.375	1.172	0.25	synergy
Tyramycin	5.000	0.625	9.375	1.172	0.25	synergy
Penicillin sodium.	40.000	20.00	9.375	0.293	0.53	additivity
Amoxicillin Sodium	4.000	2.000	9.375	0.293	0.53	additivity
Cefquinome sulfate	0.620	0.313	9.375	0.590	0.56	additivity
Amikacin sulfate	1.953	0.977	9.375	0.293	0.53	additivity
Doxycycline Hydrochloride	3.906	1.953	9.375	4.688	1.00	additivity
Chlortetracycline Hydrochloride	7.812	0.488	9.375	4.688	0.56	additivity
Oxytetracycline Hydrochloride	1.953	0.488	9.375	4.688	0.56	additivity
Erythromycin	313.000	156.000	9.375	0.293	0.53	additivity
Ampicillin Sodium	4.000	0.125	9.375	9.375	1.03	indifference

Table 2: Combined Drug Sensitivity of Some Antibiotics and Matrine to BHS.

Drug	Antibiotics（μg/mL）		Matrine（mg/mL）		FICI	Results
	MIC_A	C_A	MIC_B	C_B		
Tylosin tartrate	156.300	39.100	9.375	1.172	0.38	synergy
Tilmicosin	195.000	49.000	9.375	1.172	0. 38	synergy
Tyramycin	20.000	2.500	9.375	0.586	0.19	synergy
Penicillin sodium.	96.000	48.000	9.375	1.172	0.63	additivity
Ampicillin Sodium	96.000	48.000	9.375	0.586	0.56	additivity
Amoxicillin Sodium	192.000	96.000	9.375	4.679	0.75	additivity
Ceftiofur Sodium	32.000	16.000	9.375	0.293	0.53	additivity
Cefquinome sulfate	16.000	8.000	9.375	1.172	0.63	additivity
Streptomycin sulfate	62.500	15.625	9.375	4.687	0.75	additivity
Neomycin sulfate	62.500	0.977	9.375	4.687	0.52	additivity
Gentamicin Sulfate	3.906	1.954	9.375	4.687	1	additivity
Kanamycin Sulfate	625.000	78.125	9.375	4.687	0.63	additivity
Amikacin sulfate	3.906	0.244	9.375	4.687	0.56	additivity
Doxycycline Hydrochloride	78.125	19.531	9.375	4.688	0.56	additivity
Chlortetracycline Hydrochloride	156.250	19.531	9.375	4.688	0.63	additivity
Oxytetracycline Hydrochloride	312.500	39.062	9.375	4.688	0.63	additivity
Erythromycin	391.000	98.000	9.375	2.344	0.50	additivity

Table 3: Combined Drug Sensitivity of Some Antibiotics and Matrine to *S. paratyphi* B.

Drug	Antibiotics（μg/mL）		Matrine（mg/mL）		FIC	Results
	MIC_A	C_A	MIC_B	C_B		
Ceftiofur Sodium	12.500	1.560	9.375	1.172	0.25	synergy
Gentamicin Sulfate	3.906	0.977	9.375	0.293	0.28	synergy
Erythromycin	195.000	24.000	9.375	1.172	0.25	synergy
Tyramycin	5.000	0.625	9.375	1.172	0.25	synergy
Penicillin sodium.	64.000	8.000	9.375	4.688	0.63	additivity
Ampicillin Sodium	64.000	32.000	9.375	0.293	0.53	additivity
Amoxicillin Sodium	64.000	32.000	9.375	2.344	0.75	additivity
Cefquinome sulfate	12.500	1.563	9.375	4.688	0.63	additivity
Neomycin sulfate	62.500	31.25	9.375	1.172	0.63	additivity
Amikacin sulfate	3.906	1.953	9.375	0.293	0.53	additivity
Doxycycline Hydrochloride	39.062	19.531	9.375	1.172	0.63	additivity
Chlortetracycline Hydrochloride	78.125	4.883	9.375	4.688	0.56	additivity
Oxytetracycline Hydrochloride	156.250	9.766	9.375	4.688	0.56	additivity
Tylosin tartrate	156.300	39.100	9.375	2.344	0.5	additivity
Tilmicosin	391.000	19.500	9.375	0.293	0.53	additivity
Streptomycin sulfate	31.25	31.25	9.375	0.293	1.03	indifference
Kanamycin Sulfate	250.000	3.906	9.375	9.375	1.02	indifference

Table 4: Combined Drug Sensitivity of Some Antibiotics and Matrine to *P. multocida*.

Drug	Antibiotics（μg/mL）		Matrine（mg/mL）		FICI	Results
	MIC_A	C_A	MIC_B	C_B		
Ceftiofur Sodium	0.625	0.156	9.375	1.172	0.31	synergy
Cefquinome sulfate	0.780	0.195	9.375	2.344	0.50	synergy
Streptomycin sulfate	0.977	0.122	4.687	1.172	0.50	synergy
Gentamicin Sulfate	0.977	0.244	9.375	1.172	0.38	synergy
Kanamycin Sulfate	0.977	0.122	9.375	0.586	0.19	synergy
Amikacin sulfate	9.766	0.610	18.75	1.172	0.13	synergy
Erythromycin	391.000	49.000	2.344	0.049	0.38	synergy
Tilmicosin	48.750	12.180	4.688	0.586	0.38	synergy
Tyramycin	2.500	0.313	2.344	0.293	0.25	synergy
Penicillin sodium.	40.000	20.000	9.375	0.293	0.53	additivity
Ampicillin Sodium	10.000	5.000	9.375	4.679	1.00	additivity
Neomycin sulfate	0.977	0.488	9.375	0.586	0.56	additivity
Doxycycline Hydrochloride	3.906	0.976	9.375	4.688	0.75	additivity
Chlortetracycline Hydrochloride	7.812	7.812	9.375	4.688	1.00	additivity
Oxytetracycline Hydrochloride	3.906	0.976	9.375	4.688	0.75	additivity
Tylosin tartrate	156.300	78.100	4.688	1.172	0.75	additivity
Amoxicillin Sodium	10.000	0.312	9.375	9.375	1.03	indifference

Table 5: Combined Drug Sensitivity of Some Antibiotics and Matrine to *S. aureus*.

Drug	Antibiotics（μg/mL）		Matrine（mg/mL）		FICI	Results
	MIC_A	C_A	MIC_B	C_B		
Streptomycin sulfate	1.560	0.190	4.687	1.172	0.38	synergy
Kanamycin Sulfate	3.120	0.780	4.687	1.172	0.50	synergy
Amikacin sulfate	0.625	0.156	4.687	1.172	0.50	synergy
Tilmicosin	12.200	1.500	4.688	1.172	0.38	synergy
Tyramycin	1.000	0.250	4.688	0.293	0.31	synergy
Ceftiofur Sodium	1.250	0.625	9.375	0.293	0.53	additivity
Cefquinome sulfate	0.313	0.156	9.375	0.293	0.53	additivity
Neomycin sulfate	2.440	0.610	4.687	2.344	0.75	additivity
Gentamicin Sulfate	0.488	0.244	9.375	4.6875	1.00	additivity
Doxycycline Hydrochloride	0.310	0.150	4.688	0.586	0.63	additivity
Chlortetracycline Hydrochloride	0.244	0.122	4.688	0.293	0.56	additivity
Oxytetracycline Hydrochloride	0.488	0.244	4.688	0.586	0.63	additivity
Erythromycin	39.100	6.00	4.688	2.344	0.52	additivity
Tylosin tartrate	24.400	1.500	4.688	2.344	0.56	additivity
Penicillin sodium.	0.310	0.160	9.375	4.688	1.00	indifference
Ampicillin Sodium	0.630	0.310	9.375	4.679	1.00	indifference
Amoxicillin Sodium	0.310	0.160	9.375	4.679	1.00	indifference

reverse bacterial drug resistance (Li *et al*., 2022; Liu *et al*., 2022). Six common clinical Chinese herbal medicines including red peony root, *Qingdai*, gallnut, wild chrysanthemum, *Houttuynia cordata* and berberine are effective against MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Gallnut and berberine have demonstrated abilities to partially reverse the drug resistance in both these MDR species (Fang *et al*., 2017). Other traditional Chinese medicines such as *Coptis chinensis*, *Scutellaria baicalensis* and *Forsythia chinensis* have also exhibited varying degrees of antibacterial effects on common clinical pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *Candida albicans*. For example, *C. chinensis* and *S. baicalensis* have shown high sensitivity to *S. aureus* while *F. chinensis* is highly effective against *P. aeruginosa* (Chen and Li 2018; Hu *et al*., 2018). Furthermore, *Patchouli* spp. (Lamiaceae) has a significant inhibitory effect on drug-resistant strains of *Helicobacter pylori* (Zhong *et al*., 2021). In the current study we found that matrine exhibited a significant

antibacterial effect against 5 common clinical bacteria; *S. aureus*, BHS, *P. multocida*, *E. coli* and *S. paratyphi* B. The antibacterial effect of matrine was particularly with an MIC range of 4.688-9.375 mg/mL.

Traditional Chinese medicines have shown synergism with antibiotics, enhancing their efficacy and reducing bacterial drug resistance in clinical settings (Bao *et al*., 2020). *In vitro* studies have demonstrated that the combination of andrographolide and aminoglycosides can increase the sensitivity of drug-resistant *E. coli* primarily by eliminating drug-resistance plasmids and efflux pumps (Zhang *et al*., 2017). Baicalin has also been found to enhance the antibacterial activity of colistin (Cui *et al*., 2023), tobramycin (Jin *et al*., 2023), and doxycycline (Wang *et al*., 2023) against *E. coli*, carbapenem-resistant *P. aeruginosa* and drug-resistant *Salmonella* spp. Similarly, the combination of the traditional Chinese medicine preparation Xiyanping injection and azithromycin has shown increased antibacterial and anti-inflammatory properties in the prevention and treatment of experimental *Klebsiella pneumoniae* infections in rats (Gu *et al*., 2022). These types of combinations have also been shown to reduce bacterial drug resistance. For instance, pepper extract combined with erythromycin, ceftriaxone and levofloxacin has been shown to reduce resistance to methicillin-resistant *S. aureus* (MRSA) while quercetin and tetracycline decreased the MIC against *E. coli* 4-fold (Qu *et al*., 2019). Furthermore, the combination of cefoperazone with Qingkailing and Shuanghuanglian has demonstrated significant MIC reductions against ESBL *K. pneumoniae* (Liang *et al*., 2016). In a mouse model, a combination of chenodeoxycholic acid (CDCA) and amikacin effectively protected against a *S. aureus* infection challenge. This combination has the potential to enhance the activity of aminoglycoside antibiotics since CDCA increases the uptake of aminoglycosides in a proton motive force-dependent manner by dissipating the chemical potential and potentiates ROS generation by inhibiting superoxide dismutase activity (Cui *et al*., 2023). Our experiments investigated the effectiveness of matrine in combination with 17 selected antibiotics against 5 common clinical pathogens. We found that most combinations exhibited additive or synergistic effects and was particularly striking for the tilmicosin combination.

The mutant prevention concentration (MPC) is the minimum concentration of drug required to clinically prevent the selective enrichment and amplification of drug-resistant mutant strains. The MSW refers to the range between the MIC_{99} (99% sensitivity) and the MPC that prevents the growth of one-step drug-resistant mutants (Yang *et al*., 2022). In other words, the MPC encompasses the concentration range that can prevent the growth of wild-type bacteria from the lowest concentration to the concentration that inhibits the growth of the least sensitive strain (one-step mutant). MPC is an indicator used to assess the antibacterial activity of drugs and reflects their ability to inhibit the selection of drug-resistant mutant strains. The selection index (SI) also reflects the ability of antibacterial drugs to select drug-resistant mutant strains and can indicate the size of the MSW. A smaller SI indicates a narrower MSW that indicates a lower likelihood of producing drug-resistant mutants and has a stronger ability to inhibit the selection of drug-resistant mutants (Blondeau *et al*., 2004; Jiang *et al*., 2021; Yi *et al*., 2022). Shuanghuanglian and levofloxacin combination could reduce the MSW of levofloxacin against *S. aureus* 0.4-0.8 times, thereby decreasing levofloxacin resistance (Wang *et al*., 2012). Additionally, Shuanghuanglian combined with β-lactam antibiotics also resulted in significant reductions of the MSW. For instance, when combined with amoxicillin, the SI decreased to 5.1 that was 1/3 the value for amoxicillin alone. Similarly, when combined with ceftiofur, the SI was reduced to 4.2 and was ¼ of amoxicillin alone. The combination of *Huanglian* injection and antibiotics can also greatly enhance the ability to inhibit the emergence of drug-resistant bacterial strains (Cao, 2013).

Conclusions: Our results demonstrated that the combined use of ceftiofur, doxycycline, gentamicin, tilmicosin with matrine was effective against *E. coli*, *S. aureus*, *S. paratyphi*, *P. multocida* and BHS, surpassing the efficacy of their individual use. Moreover, these combinations significantly reduced the MSW indicating that matrine can enhance the therapeutic effect of antibiotics and mitigate bacterial resistance. Our future experiments will involve the clinical trial stage using experimental animal models to test whether the traditional Chinese medicine matrine can be clinically effective.

Acknowledgements: This work was supported by the Key Science and Technology Program of Henan Province (232102110074), Foundation of Xinyang Science and Technology (20220063), Scientific and technological Innovation team of Poultry Germplasm Resources Application and healthy breeding in Dabie Mountain of Xinyang Agriculture and Forestry University (XYKJTD-013).

Authors contribution: Haigang Wu: Methodology, Data curation, Writing-original draft, Writing-review and editing, Supervision; Mengxiao Tao: Investigation, Data curation, Writing-Original draft preparation; Jinni Liu: Data curation, Formal analysis, Writing-original draft; Xiaoquan Zhang: Investigation, Data curation; Jianxin Hu: Writing-review and editing, Supervision, Funding acquisition; Bingjie Ma: Formal analysis, Validation; Peirong Chen: Funding acquisition, Formal analysis.

Conflict of Interest: The authors declare that there is no conflict of interest.

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