International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.164.170

CrossMark

Research Article
Luteolin and Vancomycin Synergistically Resisted Methicillin-Resistant *Staphylococcus aureus*

[1]Yixuan Sun, [2]Fengjun Sun, [2]Wei Feng, [2]Qian Wang, [2]Fang Liu, [2]Peiyuan Xia and [1,2]Xuewen Qiu

[1]Department of Pharmacy, Chongqing General Hospital, Chongqing 400014, China
[2]Department of Pharmacy, The First Affiliated Hospital of Third Military Medical University (Army Medical University), Chongqing 400038, China

Abstract
Background and Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen resistant to a variety of antibiotics. This study aimed to investigate the *in vitro* antibacterial activity of luteolin combined with vancomycin against MRSA. **Materials and Methods:** Ten clinical MRSA isolates were selected and the susceptibility of MRSA to luteolin and vancomycin was tested by the K-B disc diffusion method. The Minimal Inhibitory Concentrations (MICs) were determined by the microdilution method. Based on MICs, the Fractional Inhibitory Concentration (FIC) was determined by the chessboard method. The Mutant Prevention Concentrations (MPCs) were measured by agar dilution method and the Selection Index (SI) and frequency of drug resistance was calculated. The effect of luteolin combined with vancomycin on MRSA proliferation was determined by spectrophotometry. **Results:** There were significant differences in MICs before and after the combination of luteolin and vancomycin ($p<0.05$). After the combination of luteolin and vancomycin, the FIC was mainly in the range of FIC<0.5 and 0.5<FIC<1.0, mainly showing the synergistic effect (86.7%), followed by the additive effect (13.3%). It was determined that there was no indifferent effect and antagonistic effect. Compared with those of luteolin and vancomycin alone, both the MPC and SI were decreased when luteolin was combined with vancomycin and the frequency of drug resistance also showed a downward trend, displaying significant differences ($p<0.05$). Compared with 1/4 MIC luteolin and 1/4 MIC vancomycin alone, the combination of 1/4 MIC luteolin and 1/4 MIC vancomycin significantly inhibited the proliferation and growth of MRSA ($p<0.05$). **Conclusion:** Luteolin has a certain anti-MRSA activity *in vitro*. Combined with vancomycin, luteolin can enhance the antibacterial activity of vancomycin, exert a synergistic or additive bacteriostatic effect, reduce the MPC of vancomycin alone and narrow the mutant selection window.

Key words: Luteolin, vancomycin, methicillin-resistant *Staphylococcus aureus*, combined administration, synergistic effect

Citation: Sun, Y., F. Sun, W. Feng, Q. Wang, F. Liu, P. Xia and X. Qiu, 2022. Luteolin and vancomycin synergistically resisted methicillin-resistant *Staphylococcus aureus*. Int. J. Pharmacol., 18: 164-170.

Corresponding Author: Xuewen Qiu, Department of Pharmacy, Chongqing General Hospital, Chongqing 400014, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Staphylococcus aureus (SA), a common type of Gram-positive bacteria, is a major pathogen in humans and animals, which can cause infections in the skin, soft tissues and other sites and lead to life-threatening diseases such as pneumonia and blood infection in severe cases. Methicillin-Resistant SA (MRSA) is resistant to isoxazolyl penicillins (such as methicillin, oxacillin and flucloxacillin) and cefradine or with a positive *mecA* gene. MRSA is an important pathogen causing diseases such as necrotizing pneumonia, severe septicemia and necrotizing fasciitis and is resistant to a variety of antibiotics. One of the important reasons is that MRSA is closely associated with the formation of bacterial biofilm[1,2]. In recent years, due to the abuse of antibiotics, the infection rate of MRSA is increasing gradually[3]. As a type of glycoside, vancomycin is the first choice for the treatment of MRSA infection. The cure rate of vancomycin against MRSA is 63.55%[4]. Meanwhile, the survey of Gao *et al.*[5] manifested that the cure rate of vancomycin against MRSA was 80%. However, with the extensive application of vancomycin, the minimal inhibitory concentration of vancomycin has increased in clinical practice and Vancomycin-Intermediate SA (VISA) and Vancomycin-Resistant SA (VRSA)[6] have emerged. Hence, it is necessary to explore new combined treatment schemes.

Luteolin has a variety of biological activities, such as antibacterial, antiviral, antioxidant and antitumor activities and can reduce blood lipids and cholesterol[7]. Pharmacological studies on luteolin are mainly focused on its functions, such as anti-inflammation, antitumor, immune regulation and heart protection and its influence on the respiratory system[8]. Wang *et al.*[9] studied the antibacterial activity and antibacterial mechanism of luteolin against SA and found that luteolin had obvious antibacterial activity against SA, mainly by inhibiting the activity of DNA topoisomerase and thereby affecting the synthesis of nucleic acids and proteins. So far, the research on the antibacterial activity of luteolin is mainly against SA. However, there have been no studies on the antibacterial activity of luteolin against MRSA and its mechanism. In this study, luteolin and vancomycin were combined to observe their synergistic effect against MRSA *in vitro*, to provide a new scientific basis for the clinical treatment of MRSA infection.

MATERIALS AND METHODS

Study area: This study was carried out from January, 2019 to December, 2020.

Bacterial strains: Ten MRSA strains were isolated and provided by the clinical laboratory of the hospital. All the isolated strains were identified by VITEK 32 and 16S rRNA. SA ATCC25923 (from the National Institutes for Food and Drug Control) was used as the quality control strains.

Culture medium and antibacterial drugs: The experimental materials included Tryptic Soy Broth (TSB) medium, Mueller-Hinton (MH) agar (Oxoid), drug susceptibility disks (Hangzhou Tianhe Microbial Reagent Co., Ltd.), vancomycin (Sigma, USA), 98% luteolin (Institute of Natural Medicine, Henan University), crystal violet solution (GBCBIO Technologies Inc.) and 0.5 McFarland turbidimetric tube (Shanghai Xinrui Instrument Co., Ltd.).

Main apparatus: The experimental apparatus mainly included a constant temperature incubator (Napco, USA), optical microscope (Shanghai Huxing Optical Instrument Co., Ltd.), 96-well cell culture plate (Costar, USA) as well as WalkAway-96 automatic bacterial identification and drug susceptibility analyzer (Siemens, Germany).

Preparation of bacterial suspension: After 4-5 MRSA colonies were inoculated into MH broth and enriched for 6 hrs, the turbidity of the bacterial fluid was corrected to 0.5 MacFarland standards using a turbidimeter, followed by dilution with MH broth to 1.5×10^5 CFU mL^{-1} for later use.

Drug susceptibility test against MRSA: K-B disc diffusion method was adopted to test the susceptibility of MRSA to luteolin and vancomycin. In a super clean bench, the sterilized agar medium (sterilization condition: 121.0°C, 15 min) was poured into the petri dish and the dish was labelled. Later, 500 µL of bacterial fluid at a concentration of 10^{7-8} CFU mL^{-1} was added into the dish with a micropipette and smeared evenly using a spreader to prepare a dish with bacteria. A circular filter paper (diameter: 6 mm) containing 5 µL of the sample was placed on the dish with bacteria and another circular filter paper (diameter: 6 mm) containing 5 µL of DMSO was used as the blank control. After culture in a constant temperature incubator at 37°C for 24 hrs, the diameter of the bacteriostatic zone was recorded and three parallel samples were set for each sample. All the operations were carried out under aseptic conditions. According to the WHO NET 4 NCCLS standards, the results were interpreted as R (resistant), I (intermediate) and S (susceptible) and susceptibility quality control were performed by using SA ATCC25923[10].

Determination of MICs: MICs of luteolin and vancomycin were determined by the microdilution method. Specifically, 100 µL of MH medium, 50 µL of drug solution at different concentrations and 50 µL of test bacterial fluid were added to the 96-well plate, followed by the culture at 37°C for 24 hrs. The final concentration of luteolin and vancomycin was 1,435 and 1,920 μg mL^{-1}, respectively. The wells without drugs were taken as the positive control group, while those without test bacteria were used as the negative control group. The minimal drug concentration inhibiting the growth of the test bacteria was defined as the MIC for the bacteria. MIC_{50} was defined as MIC required inhibiting the growth of 50% of the test strains and MIC_{90} was defined as MIC required inhibiting the growth of 90% of the test strains. The minimal drug concentration required in the aseptic growth dish was the MIC of the drug against the bacteria. According to the Clinical and Laboratory Standards Institute 2015 standards, the minimal drug concentration for inhibiting bacterial growth on the agar plate was MIC.

Chessboard assay for antibacterial synergy: In a sterile 96-well plate, chessboard dilution was adopted to double dilute luteolin and vancomycin in horizontal and vertical columns, respectively. 6 dilution gradients were selected for each drug, with the highest concentration of 2×MIC and the lowest of 1/16×MIC. It was ensured that there was only luteolin in the first horizontal column and vancomycin in the first vertical column. Besides, wells in the first horizontal column (positive control) were only added with bacterial fluid and nutritional broth, while wells in the first vertical column (negative control) were only added with nutritious broth. The concentration of bacteria was successively increased from left to right in the horizontal axis and successively decreased from top to bottom in the vertical axis. Luteolin (50 μL) and vancomycin (50 μL) were added into each well and then 100 μL of bacterial fluid (1.5×10^5 CFU mL^{-1}) was added. The final concentration of the bacterial fluid was 5×10^5 CFU mL^{-1}. After the sample was added, the plate was shaken, mixed on a micro oscillator and then incubated overnight in a constant temperature incubator at 37°C. Based on MICs when the two drugs were combined, the combined Fractional Inhibitory Concentration (FIC) was calculated according to the following Eq.:

$$\text{FIC} = \frac{\text{MIC}_{\text{A drug combined}}}{\text{MIC}_{\text{A drug alone}}} + \frac{\text{MIC}_{\text{B drug combined}}}{\text{MIC}_{\text{B drug alone}}}$$

- FIC$\leq$0.5 indicated synergistic effect
- 0.5<FIC$\leq$1.0 suggested additive effect
- 1.0<FIC$\leq$2.0 suggested indifferent effect
- FIC>2.0 indicated antagonistic effect

Detection of mutant prevention concentration (MPC): According to the concentration of the drug, the agar plates were divided into 1×MIC, 2×MIC... and 64×MIC groups. Glucose 6-phosphate with a concentration of 25 μg mL^{-1} was dripped into each group of agar plates and MPC of vancomycin alone was determined. Vancomycin was prepared at different concentrations. Meanwhile, RET lipoprotein was prepared at a concentration of 4.0 μg mL^{-1} and then mixed with 18 mL of MH agar and the prepared luteolin in a petri dish. After culture, the bacteria were inoculated into a fresh MH medium and then shaken and cultured in a constant temperature incubator at 37°C for 12 hrs, followed by centrifugation at 3,000 rpm min^{-1}. Later, the precipitates were preserved and mixed into the culture medium. After culture for another 6 hrs, the solution was centrifuged again with a liquid concentration at 3×10^{10} CFU mL^{-1}. The colony liquid was sucked out and smeared evenly on the labelled agar plate (1.2×10^{10} CFU per plate). After culturing at 37°C for 72 hrs, the minimal drug concentration without bacterial growth was the MPC. The Selection Index (SI) and frequency of drug resistance were calculated as follows:

$$\text{SI} = \frac{\text{MPC}}{\text{MIC}}$$

$$\begin{array}{c}\text{Frequency of}\\ \text{drug resistance}\end{array} = \frac{\text{Detected number of colonies on the agar plate}}{\text{Number of colonies inoculated in the experiment}}$$

Evaluation of effects of combined administration on the growth curve of MRSA: The ATCC25923 strains were activated and a single strain was selected and placed in 0.9% sterile normal saline. The concentration of bacterial fluid was adjusted to 0.5 McFarland standards and 0.5% inoculum was added into the MH broth medium. Four groups were set up in this experiment, namely, 1/4 MIC luteolin group, 1/4 MIC vancomycin group, 1/4 MIC luteolin+1/4 MIC vancomycin group and blank control group. The strains were cultured in a constant temperature incubator at 37°C for 24 hrs. The OD value of the sample was measured at 600 nm every 2 hrs and the growth curve of MRSA was plotted.

Statistical analysis: SPSS 18.0 software was utilized for statistical analysis and the t-test was employed to analyze the differences between groups. p<0.05 suggested that a difference was statistically significant.

RESULTS

MIC and resistance of MRSA when luteolin and vancomycin were used alone or in combination: The results of the drug susceptibility test revealed that no luteolin- and vancomycin-resistant strains were found in the 10 clinical isolates of MRSA in Table 1. The average MICs of luteolin and vancomycin against the 10 strains of MRSA were 0.138 ± 0.032 mg mL^{-1} and 1.46 ± 0.23 μg mL^{-1}, respectively. The susceptibility test results of combined drugs manifested those MICs of luteolin and vancomycin declined to 0.066 ± 0.013 mg mL^{-1} and 0.371 ± 0.036 μg mL^{-1}, respectively, after the combination of luteolin and vancomycin. There were significant differences in MICs before and after the combination of luteolin and vancomycin ($p<0.05$).

FIC distribution of luteolin combined with vancomycin against MRSA: After analyzing FIC of luteolin combined with vancomycin, it was found that the combination of luteolin and vancomycin had the synergistic effect (80%) and additive effect (20%) on MRSA. It was determined that there were no indifferent effects and antagonistic effects. FIC was mainly in the range of FIC<0.5 and 0.5<FIC<1.0 and the synergistic effect was mainly reflected, followed by the additive effect. These suggest that the combination of the two drugs had obvious antibacterial effects on MRSA in Fig. 1.

Effects of luteolin and vancomycin used alone or in combination on MPC and SI: MPC of vancomycin alone was 28.80 ± 14.70 μg mL^{-1}, while that of vancomycin combined with luteolin was 8.00 ± 3.27 μg mL^{-1} $p<0.05$. SI of vancomycin alone was 27.2 ± 7.73, while that of vancomycin in combination with luteolin was 9.20 ± 3.79. Thus, there was a significant difference in SI before and after the combination of vancomycin and luteolin ($p<0.05$) Table 2.

Effects of vancomycin combined with luteolin on resistance frequency against MRSA: The resistance frequency of vancomycin alone against MRSA was detected at concentrations of 1×MIC, 2×MIC, 4×MIC, 8×MIC, 16×MIC and 32×MIC against the 10 strains of MRSA and the results showed that it was 1.42×10^{-5}-2.67×10^{-6}, 2.38×10^{-6}-1.74×10^{-7}, 7.58×10^{-6}-5.49×10^{-7}, 3.17×10^{-7}-1.63×10^{-8}, 1.63×10^{-8}-3.44×10^{-8} and 32×MIC: 2.33×10^{-9}, respectively in Table 3.

When vancomycin was combined with luteolin, the resistance frequencies of vancomycin and luteolin were as follows: $7.78\times10^{-6}\pm6.28\times10^{-6}$ and $2.58\times10^{-8}\pm2.56\times10^{-8}$ at 1×MIC, $1.32\times10^{-6}\pm0.65\times10^{-6}$ and 1.96×100^{-8}

Table 1: Resistance of 10 MRSA strains to luteolin and vancomycin (n = 10)

Susceptibility test disk	Strain number										MIC		
	1	2	3	4	5	6	7	8	9	10	Luteolin	Vancomycin	Luteolin+vancomycin
Luteolin	S	M	S	S	S	S	S	S	S	S	0.138 ± 0.032 mg mL^{-1}	-	0.066 ± 0.013 mg mL^{-1*}
Vancomycin	S	S	S	S	M	S	S	M	S	S	-	1.46 ± 0.23 μg mL^{-1}	0.371 ± 0.036 μg $mL^{-1\#}$

M: Intermediate, MIC: Minimal inhibitory concentration, MRSA: Methicillin-resistant *Staphylococcus aureus*, R: Resistant, S: Susceptible. *Compared with MIC of luteolin alone, $p<0.05$, #Compared with vancomycin alone, $p<0.05$

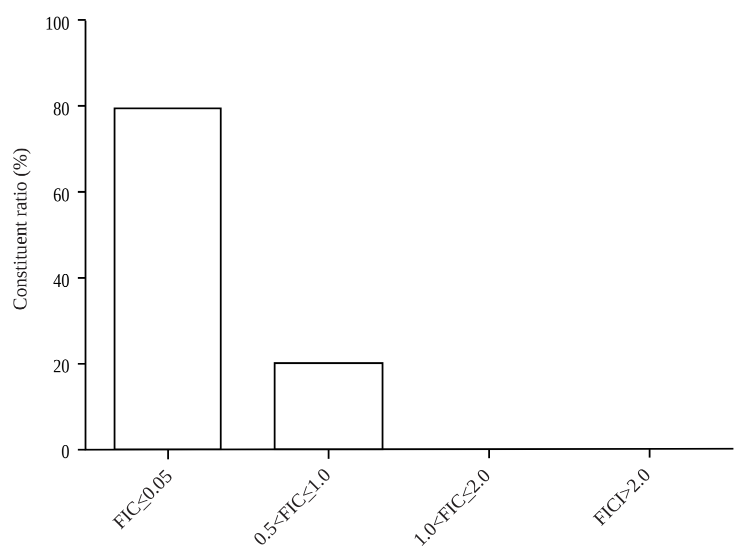

Fig. 1: FIC distribution of luteolin combined with vancomycin against 10 strains of MRSA
FIC: Fractional inhibitory concentration, MRSA: Methicillin-resistant *Staphylococcus aureus*

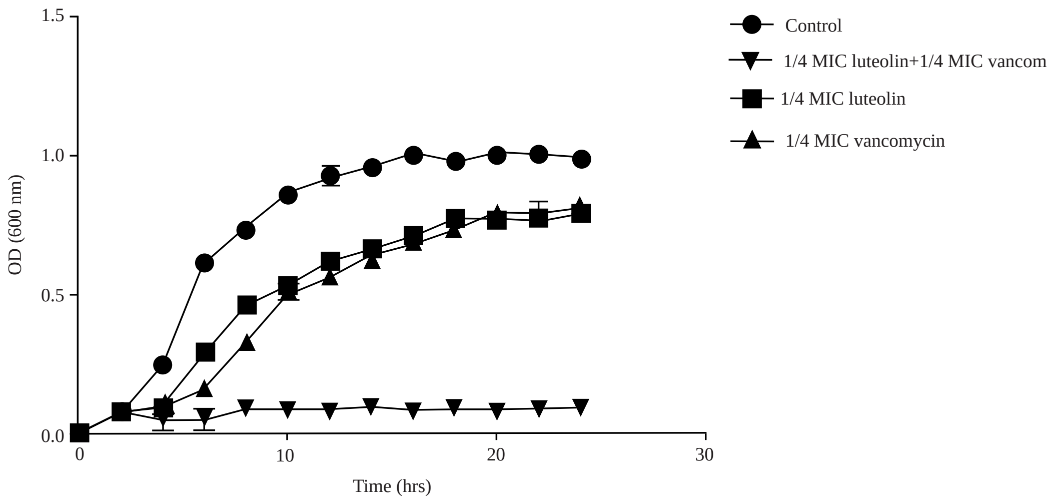

Fig. 2: Effects of luteolin combined with vancomycin on the growth curve of MRSA
MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 2: Effects of luteolin and vancomycin used alone or in combination on MPC and SI

Groups	1	2	3	4	5	6	7	8	9	10	x̄±SD
Vancomycin											
MPC (µg mL^{-1})	32	64	32	16	32	16	32	16	32	16	28.80±14.70
SI	32	32	32	16	32	16	32	16	32	32	27.20±7.73
Vancomycin+luteolin											
MPC (µg mL^{-1})	8	16	8	8	8	8	4	8	4	8	8.00±3.27
SI	8	8	8	16	8	8	4	8	8	16	9.20±3.79

MPC: Mutant prevention concentration, SI: Selection index

Table 3: Effects of vancomycin combined with luteolin on resistance frequency against MRSA

		Group	Resistance frequency
Vancomycin		1×MIC	$1.42\times10^{-5}\pm2.67\times10^{-6}$
		2×MIC	$2.38\times10^{-6}\pm0.74\times10^{-6}$
		4×MIC	$7.58\times10^{-6}\pm2.49\times10^{-6}$
Vancomycin+luteolin	Vancomycin	1×MIC	$7.78\times10^{-6}\pm6.28\times10^{-6}$
		2×MIC	$1.32\times10^{-6}\pm0.65\times10^{-6}$
		4×MIC	$4.38\times10^{-7}\pm1.66\times10^{-7}$
	Luteolin	1×MIC	$2.58\times10^{-8}\pm2.56\times10^{-8}$
		2×MIC	$1.96\times10^{-8}\pm1.86\times10^{-8}$
		4×MIC	$12.52\times10^{-9}\pm3.09\times10^{-9}$

MIC: Minimal inhibitory concentration, MRSA: Methicillin-resistant *Staphylococcus aureus*

1.86×10^{-8} at 2×MIC and $4.38\times10^{-7}\pm1.66\times10^{-7}$ and $12.52\times10^{-9}\pm3.09\times10^{-9}$ at 4×MIC, respectively. There were significant differences in the resistance frequency of vancomycin alone and that of vancomycin combined with luteolin ($p<0.05$). These suggest that the resistance frequency of vancomycin combined with luteolin was superior to that of vancomycin alone.

Effects of luteolin combined with vancomycin on the growth curve of MRSA: The effect of luteolin combined with vancomycin on the growth curve of ATCC25923 strains was determined by spectrophotometry. The growth rate of bacteria in the blank control group was higher than that in the antimicrobial groups. The growth rate of bacteria in the blank control group entered the logarithmic growth phase after 2 hrs and the stable growth phase after 10 hrs. The growth of MRSA was inhibited in varying degrees in the groups treated with 1/4 MIC luteolin and 1/4 MIC vancomycin. The bacteria in the 1/4 MIC luteolin group entered the logarithmic growth phase at 4 hrs and stable growth phase at 20 hrs, while MRSA in the 1/4 MIC vancomycin group entered the logarithmic growth phase at 6 hrs and stable growth phase at 20 hrs. Moreover, compared with those in the blank control group, the growth rate and biomass of MRSA in 1/4 MIC luteolin and 1/4 MIC vancomycin groups were decreased. After 1/4 MIC luteolin and 1/4 MIC vancomycin were combined, the growth of MRSA was inhibited and there was no proliferation within 24 hrs in Fig. 2, suggesting that luteolin can enhance the antibacterial activity of vancomycin and decrease its MIC.

DISCUSSION

With the widespread use of antibiotics, MRSA has become one of the vital pathogens of nosocomial infection. The proportion of MRSA in SA and its detection rate are increasing annually[11,12]. Vancomycin, a representative of glycopeptide antimicrobial agents, has become the first choice for the treatment of severe MRSA-induced infection worldwide[13]. Vancomycin can interfere with the cell wall synthesis stage of Gram-positive bacteria, that is, the integration of mucopeptide terminal D-Ala-D-Ala dipeptide into mitotic cells, to kill the bacteria[14]. In addition, vancomycin has the best bactericidal effect on mitotic cells. In recent years, the MIC of vancomycin against MRSA tends to increase gradually. With the widespread of MRSA and the increased application of vancomycin, vancomycin-intermediate and resistant bacteria have also emerged. Currently, the mainstay clinical schemes for the combination of vancomycin include vancomycin+rifampicin, vancomycin+aminoglycosides and vancomycin+β-lactams but hepatotoxicity and high drug resistance may occur. Hence, it is very urgent to explore effective drug combination schemes.

Plant-derived antibacterial components have broad-spectrum antibacterial activity and are not easy to produce drug resistance. Besides, they have the functions of eliminating drug-resistant plasmids, improving antibacterial efficiency and reversing bacterial drug resistance. As a result, they have attracted extensive attention[15]. Luteolin, a representative compound of flavonoids, mainly exists in natural Chinese herbal medicine such as wild chrysanthemum and honeysuckle and has anti-inflammatory, antibacterial, antitumor and antiviral activities. Studies have demonstrated that luteolin has high antibacterial activity against common pathogens such as SA, *Salmonella* and *Streptococcus*. According to studies, luteolin and apigenin with a similar structure have anti-MRSA activity. So far, the effect of luteolin and vancomycin on MRSA has been rarely reported. In this study, no luteolin- or vancomycin-resistant strains were found among the 10 MRSA strains isolated in our hospital. MICs of 30 MRSA strains treated with luteolin and vancomycin alone were 0.138 ± 0.032 mg mL^{-1} and 1.46 ± 0.23 µg mL^{-1}, respectively. After the combination of luteolin and vancomycin, MICs of vancomycin and luteolin decreased to 0.371 ± 0.036 µg mL^{-1} and 0.066 ± 0.013 mg mL^{-1}, respectively. The results of the t-test showed that there were significant differences in MICs between luteolin and vancomycin alone and the combination of luteolin and vancomycin ($p<0.05$). This suggests that a single drug has some limitations in treating MRSA infection and the *in vitro* antibacterial activity of combined drugs against MRSA is significantly higher than that of a single drug. After the combination of luteolin and vancomycin, the FIC results demonstrated that the combination of luteolin and vancomycin had a synergistic or additive effect on MRSA but no indifferent or antagonistic effect. After the combination of vancomycin and luteolin, both MIC_{50} and MIC_{90} were decreased and the doses of the two antimicrobials were reduced remarkably.

With the long-term use of vancomycin, the number of clinically isolated vancomycin-mediated and drug-resistant staphylococci has gradually increased. Therefore, researchers have endeavoured to find antibiotic sensitizers. For instance, MRSA biofilms can be effectively eliminated by D-tyrosine combined with vancomycin[16]. Combining linezolid with fosfomycin can synergistically inhibit the growth of MRSA strain *in vitro*[17]. Additionally, Zhanel *et al.*[18] found that when vancomycin was used in combination with other antibiotics, the SI of the tested strain decreased markedly. It has recently been reported that the combined use of natural drug monomers and antibiotics exerts synergistic antibacterial effects and reverses the resistance of bacteria to antibiotics. For example, cinnamaldehyde can enhance the influence of vancomycin on MRSA biofilms, showing synergistic antibacterial effects[19]. Moreover, luteolin can reverse the resistance of amoxicillin-resistant *E. coli* probably by inhibiting the activity of broad-spectrum β-lactamase[20]. The findings of this study are following those of the studies mentioned above.

According to the Mutant Selection Window (MSW) theory, when the drug concentration is within MSW, the susceptible bacteria will be killed and the resistant bacteria will survive, which leads to the selective amplification of drug-resistant mutants[21]. Hence, MPC is a crucial index for determining the resistance of bacteria to antibiotics[22,23]. In this study, after the combination of vancomycin and luteolin, both the MPC and SI were decreased compared with those of vancomycin alone and the resistance mutation frequency of MRSA showed a downward trend, which could greatly reduce the proliferative activity of MRSA.

CONCLUSION

In summary, luteolin exhibits anti-MRSA activity *in vitro*. In combination with vancomycin, luteolin can enhance the activity of vancomycin as well as reduce the MPC of vancomycin alone and the dose of antibiotics.

SIGNIFICANCE STATEMENT

This study discovers the *in vitro* antibacterial activity of luteolin combined with vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) that can be beneficial for antibacterial therapy. This study will help the researcher to uncover the critical area of synergistic antibacterial action that many researchers were not able to explore. Thus, a new theory on effective MRSA treatment may be arrived at.

REFERENCES

1. Craft, K.M., J.M. Nguyen, L.J. Berg and S.D. Townsend, 2019. Methicillin-resistant *Staphylococcus aureus* (MRSA): Antibiotic-resistance and the biofilm phenotype. MedChemComm, 10: 1231-1241.
2. Dakheel, K.H., R.A. Rahim, V.K. Neela, J.R. Al-Obaidi, T.G. Hun and K. Yusoff, 2016. Methicillin-resistant *Staphylococcus aureus* biofilms and their influence on bacterial adhesion and cohesion. BioMed Res. Int., Vol. 2016. 10.1155/2016/4708425.
3. Boswihi, S.S. and E.E. Udo, 2018. Methicillin-resistant *Staphylococcus aureus*: An update on the epidemiology, treatment options and infection control. Curr. Med. Res. Pract., 8: 18-24.
4. Chavanet, P., 2013. The ZEPHyR study: A randomized comparison of linezolid and vancomycin for MRSA pneumonia. Méd. Mal. Infectieuses, 43: 451-455.
5. Gao, Z., F. Zhang, L. Wang, J. Hang, X. Liang and Y. Fan, 2014. Application of therapeutic drug monitoring of vancomycin. Chinese J. Infec. Chemother., 6: 526-531.
6. Ghahremani, M., N.H. Jazani and Y. Sharifi, 2018. Emergence of vancomycin-intermediate and -resistant *Staphylococcus aureus* among methicillin-resistant *S. aureus* isolated from clinical specimens in the Northwest of Iran. J. Global Antimicrob. Resist., 14: 4-9.
7. Guo, Y., Y. Liu, Z. Zhang, M. Chen and D. Zhang *et al*., 2020. The antibacterial activity and mechanism of action of luteolin against *Trueperella pyogenes*. Infec. Drug Resist., 13: 1697-1711.
8. Nabavi, S.F., N. Braidy, O. Gortzi, E.S. Sanchez, M. Daglia, K.S. Wozniak and S.M. Nabavi, 2015. Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. Brain Res. Bull., 119: 1-11.
9. Wang, Q. and M. Xie, 2010. Antibacterial activity and mechanism of luteolin on *Staphylococcus aureus*. Acta Microbiol. Sin., 50: 1180-1184.
10. Wang, D., K. Xie, D. Zou, M. Meng and M. Xie, 2018. Inhibitory effects of silybin on the efflux pump of methicillin-resistant *Staphylococcus aureus*. Mol. Med. Rep., 18: 827-833.
11. Paonessa, J.R., R.D. Shah, C.I. Pickens, B.D. Lizza and H.K. Donnelly *et al*., 2019. Rapid detection of methicillin-resistant *Staphylococcus aureus* in BAL. A pilot randomized controlled trial.Chest, 155: 999-1007.
12. Looney, A.T., E.J. Redmond, N.M. Davey, P.J. Daly, C. Troy, B.F. Carey and I.M. Cullen, 2017. Methicillin-resistant *Staphylococcus aureus* as a uropathogen in an irish setting. Medicine, Vol. 96. 10.1097/md.0000000000004635.
13. Purrello, S.M., J. Garau, E. Giamarellos, T. Mazzei, F. Pea, A. Soriano and S. Stefani, 2016. Methicillin-resistant *Staphylococcus aureus* infections: A review of the currently available treatment options. J. Global Antimicrob. Resist., 7: 178-186.
14. Cong, Y., S. Yang and X. Rao, 2020. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. J. Adv. Res., 21: 169-176.
15. Li, J., D. Liu, X. Tian, S. Koseki, S. Chen, X. Ye and T. Ding, 2019. Novel antibacterial modalities against methicillin resistant *Staphylococcus aureus* derived from plants. Crit. Rev. Food Sci. Nutr., 59: S153-S161.
16. Blackman, L.D., Y. Qu, P. Cass and K.E.S. Locock, 2021. Approaches for the inhibition and elimination of microbial biofilms using macromolecular agents. Chem. Soc. Rev., 50: 1587-1616.
17. Xu-Hong, Y., M.E. Falagas, W. Dong, D.E. Karageorgopoulos, L. De-Feng and W. Rui, 2014. *In vitro* activity of fosfomycin in combination with linezolid against clinical isolates of methicillin-resistant *Staphylococcus aureus*. J. Antibiot., 67: 369-371.
18. Zhanel, G.G., M. Mayer, N. Laing and H.J. Adam, 2006. Mutant prevention concentrations of levofloxacin alone and in combination with azithromycin, ceftazidime, colistin (polymyxin E), meropenem, piperacillin-tazobactam and tobramycin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., 50: 2228-2230.
19. Jia, P., Y.J. Xue, X.J. Duan and S.H. Shao, 2011. Effect of cinnamaldehyde on biofilm formation and sarA expression by methicillin-resistant *Staphylococcus aureus*. Lett. Appl. Microbiol., 53: 409-416.
20. Eumkeb, G. and S. Chukrathok, 2013. Synergistic activity and mechanism of action of ceftazidime and apigenin combination against ceftazidime-resistant *Enterobacter cloacae*. Phytomedicine, 20: 262-269.
21. Alieva, K.N., E.N. Strukova, M.V. Golikova, Y.A. Portnoy, S.H. Zinner and A.A. Firsov, 2018. Time inside the mutant selection window as a predictor of staphylococcal resistance to linezolid. J. Antibiot., 71: 514-521.
22. Lozano Huntelman, N.A., N. Singh, A. Valencia, P. Mira and M. Sakayan *et al*., 2020. Evolution of antibiotic cross resistance and collateral sensitivity in *Staphylococcus* epidermidis using the mutant prevention concentration and the mutant selection window. Evol. Appl., 13: 808-823.
23. Gianvecchio, C., N.A. Lozano, C. Henderson, P. Kalhori and A. Bullivant *et al*., 2019. Variation in mutant prevention concentrations. Front. Microbiol., Vol. 10. 10.3389/fmicb.2019.00042.