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# Antibacterial synergy between linezolid and baicalein against methicillin-resistant *Staphylococcus aureus* biofilm *in vivo*

Tangjuan Liu [a], Jing Luo [a], Guan Bi [b], Zhongye Du [a], Jinliang Kong [a,**], Yiqiang Chen [a,*]

[a] Department of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Nanning, China
[b] Department of Intensive Care Unit, The Second Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Nanning, China

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) can form biofilms, which prevents the penetration of antibiotics, decreasing their efficacy. This study investigated whether baicalein has synergistic antibacterial effects with linezolid *in vivo*. We cultivated MRSA 17546 biofilms on silicone implants and inserted them into the air pouches of rat models. The rats were treated with linezolid, baicalein, or a combination therapy for three consecutive days. All treatments reduced the number of colony-forming units (CFU) in the biofilms compared to the control ($p < 0.05$). However, by day two, the CFU counts were significantly lower in the combination group than in the individual treatment groups ($p < 0.05$). Histological analysis of the air pouches showed that the severity of the inflammatory cell infiltration was severe in the combination therapy group. In the combination group, the biofilm structure on the implant's surface was sparse and more free colonies could be seen by scanning electron microscopy (SEM); by day three, no obvious biofilm was observed. The serum levels of *Staphylococcus* enterotoxin A (SEA), C-reactive protein (CRP), and procalcitonin (PCT) were the lowest in the group where rats were treated with the combination of baicalein and linezolid ($p < 0.05$) compared to other groups. The results suggest that baicalein may inhibit the accessory gene regulator system, reducing the expression of SEA, thus lowering CRP and PCT levels. Furthermore, the inhibitory effect was more pronounced when baicalein was combined with linezolid. These results provide an important basis for the development of a new combination regimen to treat patients with biofilm-associated MRSA infections.

## 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen responsible for nosocomial infections, community-acquired pulmonary infections, and many catheter-related infections [1]. MRSA infections are associated with high morbidity and mortality because of biofilm (BF) formation on the surface of biomaterials, which causes great difficulties in clinical treatment for assorted reasons. In 2014, the Agency for Healthcare Research & Quality reported that MRSA had caused 23,000 deaths in the United States alone [2].

BF forms, the transition from a planktonic to a BF growth pattern, involves a variety of mechanisms comprising bacterial adhesion to the surface, growth and gathering of the microflora, and secretion of extracellular polymeric substances that form the mature BF structure [3]. BF formation enhances antibiotic resistance by inhibiting drug penetration and host immune response and leads to the intermittent release of planktonic bacteria that propagate continually, causing chronic persistent infections [4].

As we all know, vancomycin is one frontline antibiotic used to treat MRSA pneumonia [5]. However, the emergence of resistance to vancomycin is the most feared phenomenon. Therefore, more and more studies have begun to use vancomycin, linezolid or daptomycin in combination with other drugs against MRSA [6]. Linezolid (Fig. 1A) [2] is a synthetic oxazolidinone antimicrobial agent with activity against gram-positive organisms [7,8], including MRSA [8], vancomycin-resistant MRSA [2,9], and vancomycin-resistant *Enterococci* (VRE) [10]. Linezolid has been suggested to have better lung tissue penetration than vancomycin [11]. It has been reported that linezolid has inhibitory effects against MRSA-BF infection [12]. However, dose-dependent thrombocytopenia may be caused by high-dosage of linezolid [13]. One study reported that combinations of linezolidone with doxycycline, fosfomycin, levofloxacin, rifampicin or vancomycin

* Corresponding author.
** Corresponding author.
*E-mail addresses:* kjl071@126.com (J. Kong), chenyiq0708@163.com (Y. Chen).

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have good synergistic effects on methicillin-susceptible *Staphylococcus aureus* (MSSA) infection *in vitro* [14]. Further, the synergistic effect of linezolid and fosfomycin against MRSA-BF infection has been observed both *in vitro* and *in vivo* [1,15], providing an important clinical treatment strategy.

Baicalein (5, 6, 7-trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one) (Fig. 1B) [16,17], a major active flavonoid found in the roots of *Scutellaria baicalensis* Georgi and used in traditional Chinese medicine (TCM), displays low toxicity in humans. Baicalein is widely used to treat fever, upper respiratory infections, and other diseases. It has many therapeutic effects, including antioxidant, anti-inflammatory, anti-tumor, and anti-fibrosis properties, among others [18–21]. Moreover, it has a significant inhibitory effect on pathogens, fungi, and viruses [22,23]. In our earlier research, we explored whether baicalein had an inhibitory effect on *Pseudomonas aeruginosa* (*P. aeruginosa*) BFs and whether it had synergistic effects with levofloxacin, tobramycin or ceftazidime against *P. aeruginosa* BF infection *in vitro* and *in vivo* [17]. The results revealed the potential of baicalein for treating *P. aeruginosa* infection. It demonstrated strong antibacterial and anti-inflammatory activity. Furthermore, we demonstrated the destructive and sterilizing effect on *Aspergillus fumigatus* BF by combining baicalein with amphotericin B. Our prophase study [24] showed that baicalein inhibits MRSA early, thus disrupting the quorum sensing system and preventing mature BF formation *in vitro*. The results also suggested that combining baicalein with levofloxacin and vancomycin improves the therapeutic effects on MRSA-BF-associated infections *in vitro*.

Considering the previous findings, we believe that baicalein may have the potential for effective treatment of MRSA-BF-related infections *in vivo*. Our research team has studied the synergistic effect of vancomycin or linezolid combined with the active ingredients of scutellaria baicalensis on MRSA, which was similar. Now one of the results about vancomycin combined with the active ingredients of scutellaria baicalensis on MRSA have been written by another researcher in our research group, which is reviewing. In this study, we aimed to establish the anti-BF properties of baicalein *in vivo*, and to explore its potential synergistic effect with linezolid. Furthermore, we analyzed this synergistic effect by observing the BF morphology and the infected tissue histopathology.

## 2. Materials and methods

### 2.1. Bacterial strain and culturing

MRSA 17546, of the 21 clinical isolates sequenced *Staphylococcus aureus* (*S. aureus*) strains in our preliminary work, was selected for its strongest ability to form biofilms [24,25]. The standard strain *S. aureus* ATCC 29213 was served as a control. MRSA 17546 was pre-cultured in Luria-Bertani (LB) broth (Sigma-Aldrich, St Louis, MO, USA) at 37 °C for 20 h before use in the BF formation assays. The cultures were diluted with LB broth to a concentration of $1 \times 10^7$ colony-forming units (CFU)/milliliter (mL). MRSA-BFs were grown on implants (round medical silicone films, $r = 0.5$ centimeter (cm)). Each implant was placed into a well of a 12-well plate containing 2.5 mL of $1 \times 10^7$ CFU/mL bacteria and cultured for three days at 37 °C [25,26]. Our preliminary experimental results suggested that a relatively mature biofilm has been formed on the implant on the third day *in vitro* by scanning electron microscopy (SEM) (Supplementary information 1).

### 2.2. Reagents and antimicrobial agents

Baicalein was purchased from Sigma-Aldrich (St Louis, MO, USA, NO. 465119), and tested by high-performance liquid chromatography (HPLC) to confirm purity at > 98%. The baicalein was dissolved in dimethyl sulfoxide (DMSO) (St Louis, MO, USA) before use. Linezolid was purchased from Sigma-Aldrich (St Louis, MO, USA, NO. PZ0014). 1% pelltobarbitalum natricum, and the enzyme-linked immunosorbent assay (ELISA) kits were purchased from Sigma.

### 2.3. Rats

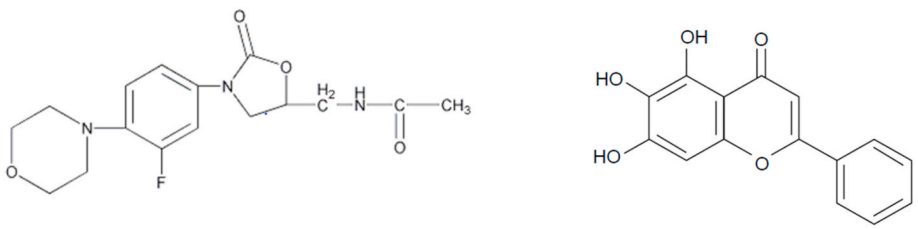
Male Sprague–Dawley (SD) rats, aged six - eight weeks, weighing 200 gram(g) - 250 g (Laboratory Animal Center of Guangxi Medical University, Guangxi, China), were acclimated for five - seven days prior to use, in an environment with controlled temperature and relative humidity, and 12-h light - dark cycles. The Guangxi Medical University Institutional Animal Care and Use Committee approved all animal studies and experimental protocols.

### 2.4. Induction of biofilm infections using implants in rat models

Rats were anesthetized by intraperitoneal injection (i.p.) with 1% pelltobarbitalum natricum. After trimming the hair with clippers, pouches were created in the infrascapular area with a 21-gauge needle and 10 mL sterile air [27]. The implants were washed with sterile physiological saline and inserted in the air pouch [1,3,28]. The rats were divided randomly into four groups: the linezolid group (40 milligram (mg)/kilogram (kg)/every 12 hours (q12 h), i.p.), baicalein group (100 mg/kg/q12 h, i.p.), linezolid + baicalein group (linezolid 40 mg/kg/q12 h plus baicalein 100 mg/kg/q12 h, i.p.), and control group (DMSO, 0.15 mL/q12 h, i.p.) (n = 24/group: eight rats per group are sacrificed each day, six for CFU and two for histology and SEM). The rats were treated for three days after MRSA-BF implant infection. The dosages of linezolid and baicalein were chosen from our preliminary study, which tested three concentrations of each drug (linezolid (1): 20 mg/kg, 40 mg/kg, and 80 mg/kg; baicalein: 75 mg/kg, 100 mg/kg, and 200 mg/kg). Each day, after treatment, the implants were removed from the air pouches and bacterial counts of the BFs were taken for all groups.

### 2.5. The MRSA colony counts of implants surface

Pelltobarbitalum natricum (1%) was administered to anesthetize SD rats. After disinfecting the local area of skin with 75% alcohol, the implant was taken out of the air pouch, and the planktonic bacterium was removed from the surface of the implant with sterile saline. The implant was then put into a tube, containing 10 mL of sterile physiological saline, which was shaken in a 180 W (W) ultrasonic system for 10 min sonication and mixed for 10 min using a vortex shaker mixer, to




Linezolid Baicalein

**Fig. 1.** Structures of linezolid (A) and baicalein (B).

allow the bacteria to fall off and disperse. The original bacterial suspension was serially diluted into different concentrations with sterile physiological saline. Twenty microliters of each diluted bacterial suspension was spread with a sterile triangular coating bar on the LB agar plates, and incubated at 37 °C for 24 h. Log10 CFU/mL was used to express the counts of bacterial colonies.

### 2.6. Histology

Each day, after the completion of the antimicrobial therapy, the excised pouch tissues of two rats, in each group, were fixed with 10% formalin and embedded into paraffin wax blocks. Sections were stained with hematoxylin and eosin (HE stains). The scoring of tissue inflammation (grade 0, not detected; grade 1, minimal; grade 2, mild; grade 3, moderate; and grade 4, severe) was based on the number, type (neutrophils, macrophages, and lymphocytes), and location of the infiltrating cells [29].

### 2.7. Scanning electron microscopy

BF formation in rats was confirmed by SEM as previously described [27]. Each day after completion of antimicrobial therapy, at the same time as the histology assessments, two implants were carefully extracted. Each BF implant was washed with phosphate buffer solution (PBS) then immediately processed for SEM by fixing at 37 °C with 2.5% glutaraldehyde for 24 h. The next day, each implant was rinsed three times in fresh PBS (pH 7.4) for 15 min, and then an ethanol gradient (30 and 50% for 25 min, then 70, 80, and 90% for 20 min) was applied. The implants were dehydrated by rinsing them with 100% ethanol three times (the first two times for 20 min, the third time for 25 min). Finally, they were dried, coated with gold, and examined by SEM.

### 2.8. ELISA to detect serum levels of Staphylococcus enterotoxin (SE) A (SEA), C-reactive protein (CRP), and procalcitonin (PCT)

After treatment for two days, the blood was collected from the abdominal aorta of six SD rats in each group; the serum and plasma were isolated by centrifugation. The levels of SEA, CRP, and PCT were detected by ELISA.

### 2.9. Statistical analysis

Data were analyzed using SPSS 20 (SPSS, Inc., IBM Software-Armonk, NY, USA) and are expressed as the mean ± standard deviation (SD). Differences between groups were evaluated using the Chi-square test and analysis of variance (ANOVA), $p < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Synergistic effect of linezolid and baicalein on MRSA-BF colony counts in vivo

MRSA-BF-coated implants were inserted into an air pouch of rat models. Each day, after completion of the antimicrobial therapy, the implants were removed from the air pouches and bacterial counts were taken. The colony counts in the BF of the control, linezolid, baicalein, and linezolid plus baicalein groups were, respectively, 6.42 ± 0.49, 5.63 ± 0.53, 5.31 ± 0.28, and 5.00 ± 0.60 (log(CFU/mL); Fig. 2A) on day one after infection; 5.96 ± 0.41, 5.09 ± 0.47, 4.54 ± 0.80, and 3.26 ± 0.48 (log(CFU/mL); Fig. 2B) on day two after infection; and 5.21 ± 1.09, 4.19 ± 0.56, 4.11 ± 0.42, and 2.32 ± 1.79 (log(CFU/mL); Fig. 2C) on day three after infection. The linezolid, baicalein, and linezolid plus (+) baicalein groups showed lesser colony counts compared to the control group ($p < 0.05$), especially after day two of treatment. Furthermore, the linezolid plus baicalein group showed significantly lower colony numbers than the linezolid group ($p < 0.05$), but not the baicalein group ($p > 0.05$) after one day of treatment. However, by day two, the combination therapy group had significantly lower colony numbers compared to the two individual therapy groups; this trend was maintained through day three. These CFU findings show that combination treatment with linezolid and baicalein is effective against MRSA infection.

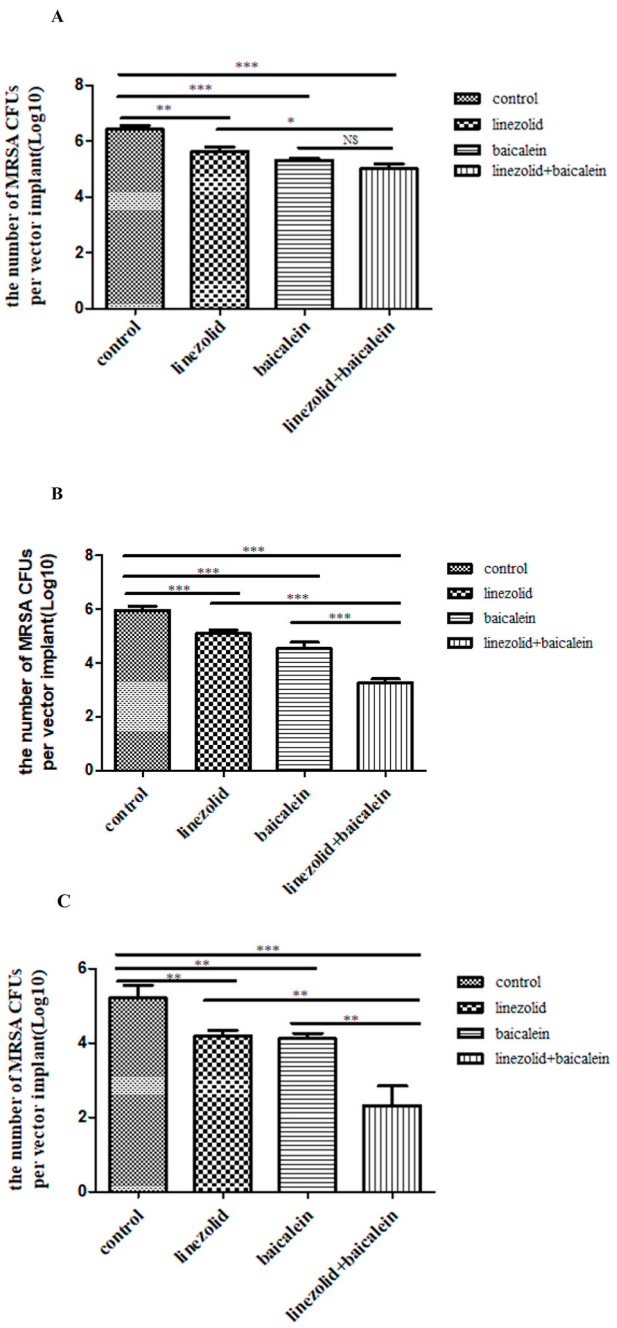



**Fig. 2.** Synergistic effect of linezolid and baicalein on MRSA biofilm colony counts *in vivo*. Colony counts from the implant biofilms of the control, linezolid, baicalein, and linezolid plus baicalein groups one day after infection (A), two days after infection (B), and three days after infection (C). Data are expressed as the mean ± SD. ***$p < 0.0005$, **$p < 0.005$, *$p < 0.05$, NS; $p > 0.05$.

### 3.2. Histological observation of the synergistic effect of linezolid and baicalein on MRSA-BF infection in vivo

The infected air pouch tissues were excised from the rats three days

after infection. As the infection progressed, the amount of inflammatory exudate increased gradually in the control group. However, exudate was lesser in the treatment groups than in the control group, especially in the linezolid plus baicalein group which had almost no exudation. The histology analysis was performed by HE staining; representative pictures of one rat from each group are shown in Fig. 3. In the pouch tissues from the control-treated (Fig. 3A1, A2, A3), linezolid-treated (Fig. 3B1, B2, B3), baicalein-treated (Fig. 3C1, C2, C3), and linezolid plus (+) baicalein-treated rats (Fig. 3D1, D2, D3) there were higher numbers of inflammatory cells, such as neutrophils and macrophages. In contrast, the tissues from the linezolid plus baicalein-treated rats showed very few neutrophils, suggesting a synergistic anti-inflammatory effect. These histological findings indicate that combination therapy with linezolid and baicalein attenuates the inflammatory reactions associated with MRSA infection.

### 3.3. SEM analysis of the synergistic effects of linezolid and baicalein on MRSA-BF infection in vivo

To examine the effects of the drugs on the structure and extent of the MRSA infection, the implants were scanned using electron microscopy (Fig. 4). The scans show the complicated fibrous structures, cells, and bacterial colonies found on the implants of the control (Fig. 4A1, A2, A3), linezolid (Fig. 4B1, B2, B3), and baicalein group (Fig. 4C1, C2, C3). A prominent reduction in the density of adherent bacteria and BF structures was observed in the scanning electron micrograph from the linezolid plus baicalein-treated rats (Fig. 4D1, D2, D3). The BF became sparser as treatment progressed.

### 3.4. Linezolid and baicalein synergistically inhibit the accessory gene regulator system and simultaneously reduce markers of inflammation

Our previous study [25] has found that baicalein can affect the SEA level of MRSA in *vitro*. CRP and PCT are the most common inflammation indicators *in vivo*. So in this study, we chose the levels of SEA, CRP and PCT to detect whether they were suppressed. After two days of drug intervention, the blood was collected from the abdominal aorta of SD rats in each group and the serum concentrations of SEA, CRP, and PCT were measured by ELISA. The serum concentrations of SEA (Fig. 5), CRP (Fig. 6), and PCT (Fig. 7) were lower in the baicalein group than in the control group ($p < 0.05$). However, the baicalein plus (+) linezolid group had the lowest levels of all three proteins ($p < 0.05$). In the linezolid group, the serum concentration of SEA was lower than that in the control group ($p < 0.05$), but there was no significant decrease in CRP and PCT levels ($p > 0.05$).

## 4. Discussion

MRSA is a clinically relevant pathogen [5,30–32] with the ability to form BF [6,33], which is responsible for antimicrobial resistance and evasion of the host immune system. Despite prevention, many BF-associated infections, particularly catheter-related BF infections, occur in clinical. Once a BF has been formed, eradication is difficult with conventional doses of antibiotics. Chinese herbal drugs [34,35] may serve as sources of new antimicrobial agents or, in combination with conventional antibiotics, may complement conventional therapies and so offer a promising strategy to overcome bacterial resistance mechanisms and restore the effectiveness of antibiotics. Inappropriate antibiotic treatment and excessive use of antibiotics, coupled with the lack of novel and more effective drugs, have greatly promoted the emergence

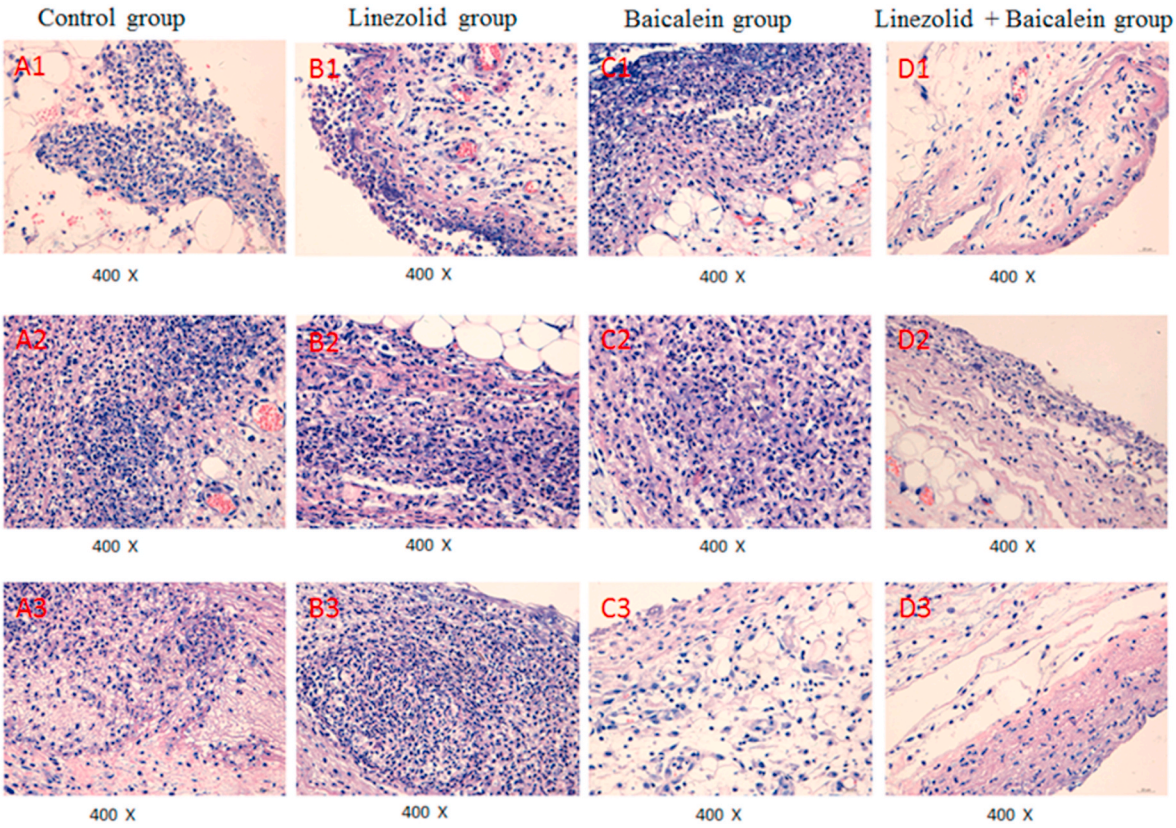



**Fig. 3.** Histological analysis of the synergistic effect of linezolid and baicalein on MRSA biofilm infection *in vivo*. Histological analysis of the pouch tissues from the control-treated (A1, A2, A3), linezolid-treated (B1, B2, B3), baicalein-treated (C1, C2, C3), and linezolid plus (+) baicalein-treated (D1, D2, D3) rats, on days one, two, and three of treatment, respectively.

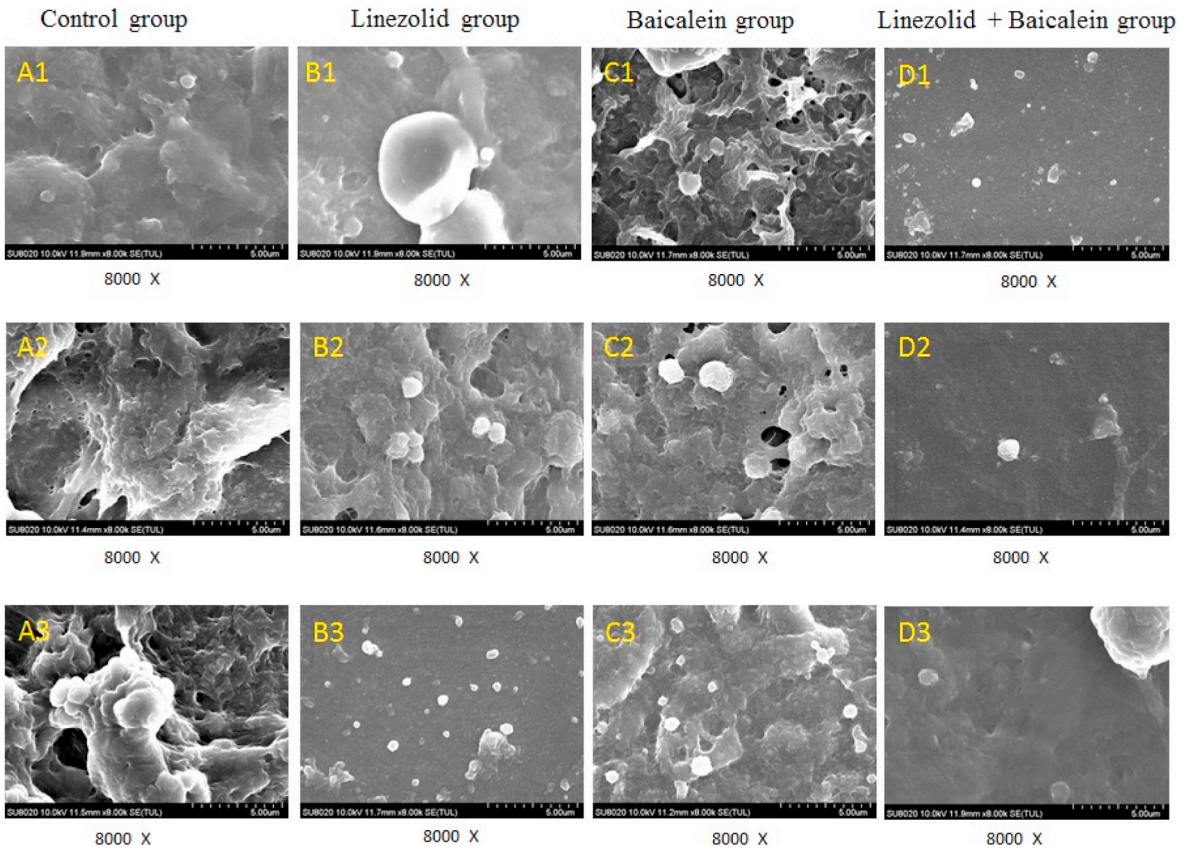



**Fig. 4.** SEM analysis of the synergistic effect of linezolid and baicalein on MRSA biofilms *in vivo*. SEM micrographs of the complicated fibrous structures, cells and colonies of bacteria forming the biofilms of the control-treated (A1, A2, A3), linezolid-treated (B1, B2, B3), baicalein-treated (C1, C2, C3), and linezolid + baicalein-treated group (D1, D2, D3), on days one, two, and three of treatment, respectively.

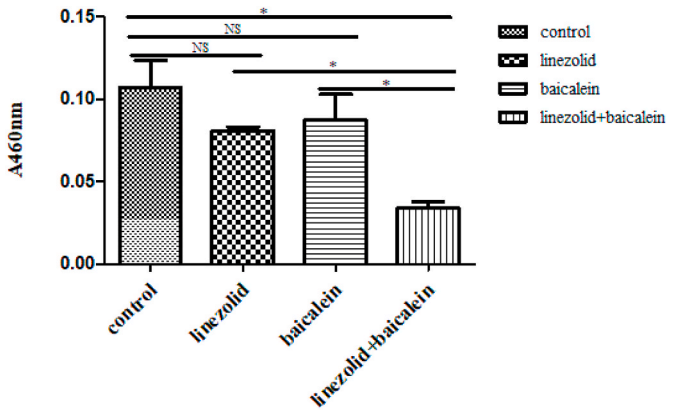



**Fig. 5.** Relative comparison of the mean serum SEA value for each drug intervention group. The relative amount of SEA in the blood of the rats from the different treatment groups (n = 6) was measured using ELISA. The intensity of the signal was measured at OD 460 nm. The results are expressed as the mean ± SD. $^{**}p < 0.005$; $^{*}p < 0.05$; NS, $p > 0.05$.

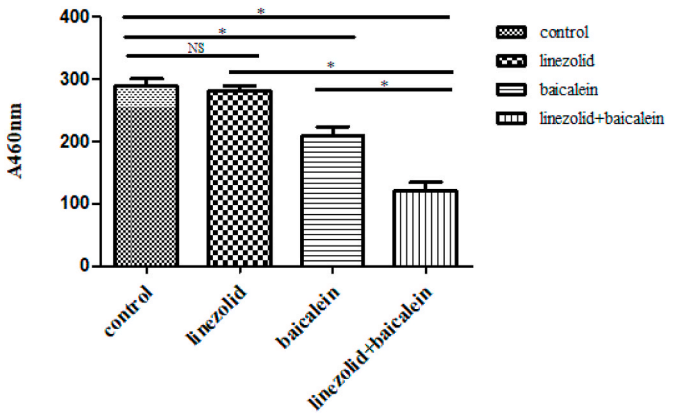



**Fig. 6.** The mean CRP concentration in the blood of the rats from each drug intervention group. The CRP blood concentration of the rats from the different treatment groups (n = 6) was measured using ELISA. The results are expressed as the mean ± SD. $^{**}p < 0.005$; $^{*}p < 0.05$; NS, $p > 0.05$.

of multi-drug resistant bacteria. Chinese herbal drugs may be used as a source of new antibacterial agents, or used in combination with conventional antibiotics to supplement conventional therapies, thus providing a promising strategy to overcome bacterial resistance mechanisms and restore the effectiveness of drugs. In our preliminary studies, baicalein was shown to have synergistic effects with various antibiotics against MRSA-BF infection *in vitro* [24]. Additionally, it has synergistic effects with various compounds against *P. aeruginosa* BF infection *in vitro* and *in vivo* [17].

To our knowledge, vancomycin and linezolid are two frontline antibiotics for the treatment of MRSA pneumonia [5,36]. MRSA was found to be sensitive to baicalein [24], although to a lesser extent than the conventional anti-MRSA antibiotics vancomycin and linezolid. In particular, there are fewer related experiments *in vivo*. The aim of this study was to investigate the *in vivo* efficacy of the combined use of linezolid and baicalein to treat MRSA infection induced by BF-coated implants inserted in the air pouches of rat models [25]. Our research showed that, in addition to its weak antibacterial effect on MRSA-BF,

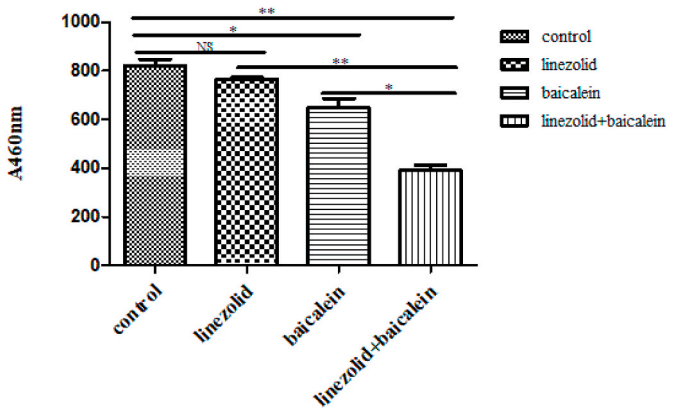



**Fig. 7.** The mean PCT concentration in the blood of the rats from each drug intervention group. The PCT blood concentration of the rats from the different treatment groups (n = 6) was measured using ELISA. The results are expressed as the mean ± SD. $^{**}p < 0.005$; $^{*}p < 0.05$; NS, $p > 0.05$.

baicalein also had the potential to enhance the activity of linezolid against MRSA-BF *in vivo*. Baicalein alone may inhibit the accessory gene regulator (Agr) system [24], reduce the expression of SEA, and thus reduce CRP and PCT serum levels. However, the inhibitory effect was more pronounced when baicalein was combined with linezolid.

The accurate prediction of synergy between linezolid and baicalein, based on the results of *in vitro* testing, is crucial to determine the optimal dosage for combined antimicrobial therapy. In our preliminary study, we used different doses of linezolid (Supplementary information 2) and baicalein (Supplementary information 3) to treat the MRSA-BF infection rats. The high-dose treatment group had high mortality, while the low-dose treatment group had poor efficacy. Thus, we chose a moderate drug combination dose for this study (linezolid 40 mg/kg i.p. plus baicalein 100 mg/kg i.p.). In this study, the most effective antibacterial activity against MRSA-BFs *in vivo* was observed with the combination therapy; linezolid and baicalein worked synergistically and their benefits were observed as early as the second day of treatment. As we know, high concentrations of antibiotics may be necessary to treat BF-related infections. However, previous studies have reported that linezolid has serious side effects, including linezolid concentration-dependent thrombocytopenia and anemia [13,37,38]. Previous *in vivo* studies of MRSA reported the impact of atorvastatin and linezolid, individually or in combination, in white rabbits with MRSA-induced pneumonia with or without mechanical ventilation [39]. The effects of fosfomycin and linezolid, individually or in combination with rifampin, against MRSA-BF infection were evaluated in guinea pigs with cage implant infections [40,41]. The antimicrobial agents could not individually eradicate the MRSA-BF, contained in the cages. The report found linezolid plus rifampin had a 50–60% cure rate, and fosfomycin plus rifampin achieved 83% cure rate; similar results were reported in another paper [1]. Therefore, combination of linezolid and fosfomycin treatment improved the therapeutic effects in BF-embedded MRSA infections.

As major constituents of Scutellariae baicalensis radix, a well-known chlorogenic acid, tanreqing (TRQ), baicalin or baicalein from the active ingredients of Chinese herbal drugs used in the treatment of inflammation, bacterial and viral infections, which has been studied extensively [42]. Chlorogenic acid was found to be effective against Gram-negative and Gram-positive bacteria (including MRSA), showing anti-staphylococcal properties against plankton and biofilm cells. They have been shown to disrupt membrane function by increasing cell membrane permeability, depolarizing the cell membrane and reducing respiratory activity, thereby exerting antibacterial effects, leading to cell death [43,44]. Yang et al. provided the first *in vitro* evidence on the synergistic effects of TRQ and vancomycin or linezolid against planktonic and biofilm MRSA, and revealed their optimal combination doses, thereby providing a rational basis for the combination therapies against MRSA [6]. Luo (one of our study group members) et al. published that baicalein attenuated the quorum sensing-controlled virulence factors of *P. aeruginosa* and relieved the inflammatory response in *P. aeruginosa*-infected macrophages by downregulating the mitogen-activated protein kinase (MAPK) and NFkappaB signal-transduction pathways [17]. And he found baicalin inhibited biofilm formation, attenuated the quorum sensing-controlled virulence and enhanced *P. aeruginosa* clearance in a mouse peritoneal implant infection model [45]. Du (one of our study group members) et al. discovered the combination effects of baicalin with levofloxacin against biofilm-related infections [46]. Recently, Chen (one of our study group members) et al. reported that baicalein inhibited MRSA biofilm formation, demonstrated biofilms, enhanced the permeability of vancomycin, reduced the production of staphylococcal enterotoxin A and α-hemolysin, and inhibited the quorum sensing system [24]. Our research team has used chlorogenic acid, baicalin or baicalein in combination with different antibiotics to treat MRSA or *P. aeruginosa*, which suggested similar synergistic bacteriostasis or biofilm formation. I have been studying the anti-MRSA effect of baicalein *in vitro*. So based on our research *in vitro* and baicalein's low toxicity, we speculated that baicalein combined with linezolid could play a synergistic bactericidal effect *in vivo* through Agr pathway, which may lead to a decrease in SEA. Now one of the results about vancomycin combined with the active ingredients of scutellaria baicalensis on MRSA have been written by another researcher in our research group, which is reviewing.

The pathogenicity of *Staphylococcus aureus* (*S. aureus)* is related to various virulence factors [47]. Thermostable SE produced by enterotoxigenic *S. aureus* strains are considered a major cause of global food poisoning, can cause foodborne disease outbreaks, and superantigen toxin reactions in human diseases [48]. *S. aureus* relies mainly on virulence factors during its growth period to establish and maintain the infection [49]. The Agr system is one of the most important and well-defined operons in *S. aureus* playing a key role in the control and regulation of virulence gene expression, which related to SEA [50–54]. In our preliminary study, the MRSA bacterial suspensions were treated with either 32 microgram (μg)/mL or 64 μg/mL baicalein for 5 h, the transcription levels of AgrA, SarA, and ICA statistically declined, regardless of whether they were normalized by 16S rRNA, GyrA or GMK [24]. In this study, the results demonstrated that baicalein in combination with linezolid has a significant synergistic antibacterial effect on MRSA-BF-infected rats, and the synergistic antibacterial effect is most pronounced on the second day of treatment. As expected, after two days of drug intervention, the MRSA virulence factor SEA, in the baicalein and linezolid group, was lower than that in the control group. SEA showed the lowest levels in the combination of the baicalein with linezolid group than in other groups. This shows that baicalein may inhibit the Agr system of MRSA and reduce its regulation of SEA. Baicalein combined with linezolid has a more pronounced inhibitory effect on the Agr system of MRSA, thereby reducing markers of inflammation, such as CRP and PCT. These results confirmed the synergistic bactericidal effect. Verma AK et al. reported that linezolid treatment ameliorates alpha-toxin-induced acute lung damage during postinfluenza community-associated MRSA infection [5]. More and more virulence factors of MRSA are being studied. The virulence factors and inflammatory factors, we measured are few, and its interaction mechanism did not really reach the molecular level. We can determine more virulence factors and verification factors in the future, and study the mechanism of action at the molecular level.

The results of the pathology and SEM analyzes confirmed the synergistic effect of the combination treatment. The synergistic effect of linezolid and baicalein was significant against the young MRSA-BF. Almost no bacteria or BF structures were observed by SEM after three days of treatment. In our study, the optimal synergistic effects of the combination therapy were observed after two days of treatment. A

synergistic effect was observed between linezolid and fosfomycin after five days of treatment demonstrating the effect on mature BF [14]. Therefore, it could be assumed that the combination of linezolid and baicalein therapy would be effective against mature MRSA-BF infections. Further work is required to confirm this theory.

Of course, our research does not in-depth study the molecular mechanisms of its synergistic effects *in vivo*. We need to detect more about virulence or inflammatory factor and do more about the mechanism of their interaction in the future study. We can study the synergy of more antibiotics combined with the active ingredients of TCM ingredients.

## 5. Conclusions

We demonstrated that the baicalein plus linezolid combination has enhanced effects against MRSA-BFs *in vivo* using implant bacterial counts, histological staining, and morphological studies. Baicalein alone may inhibit the Agr system, reduce the expression of SEA, and thus reduce CRP and PCT serum levels. However, the inhibitory effect could be more pronounced when baicalein was combined with linezolid. These results provide an important basis for the development of new regimens to treat patients with BF-associated MRSA infections, especially from implant-related infections.

## Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

## Declaration of competing interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2020.104411.

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