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Original Article

Antimicrobial effects of compound Huangbai liquid on *staphylococcus aureus, Escherichia coli* and MRSA: clinical, bacterial, and animal experimental studies

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Abstract: Objective: The aim of this study was to systematically evaluate the antimicrobial potential of Compound Huangbai Liquid (HB) against *Staphylococcus aureus* (*S. aureus), Escherichia coli* (*E. coli)* and methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Methods: A multidimensional research strategy combining clinical trials, bacteriological experiments and animal model studies was used. The clinical efficacy and adverse effects of HB were observed in patients with abdominal abscesses and non-lactating breast abscesses. The antimicrobial activity of HB was assessed in vitro by minimal inhibitory concentration (MIC), and its antibacterial, anti-inflammatory as well as pro-healing mechanisms were explored in a mouse infection model. Results: Clinical studies of abdominal abscesses showed that patients in the HB treatment group had a significantly lower rate of pus bacterial positivity, lower serum white blood cell (WBC), neutrophil (NEU) and C-reactive protein (CRP) levels, higher levels of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and basic fibroblast growth factor (βFGF), reduced pus volume and shorter hospitalization time. Clinical studies of non-lactating breast abscesses showed that HB significantly reduced tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels, promoted wound healing and relieved pain. No significant adverse effects were observed in either clinical study, and there was no statistically significant difference in the recurrence rate during long-term follow-up, indicating that HB has a favorable safety profile and stable efficacy. The results of bacteriological experiments showed that the MICs of HB on *S. aureus* and *E. coli* were 140 μg/mL and 500 μg/mL, respectively, showing concentration-dependent bacteriostatic effects. Animal experiments further revealed that HB significantly reduced the wound bacterial load, inhibited inflammatory cell infiltration, increased the number of fibroblasts, and down-regulated the levels of inflammatory factors such as IL-1, IL-6, and TNF-α, while up-regulating the expression of tissue repair factors such as EGF, VEGF-A, and TGF-β. Conclusion: HB possesses antimicrobial effects against *S. aureus* and *E. coli* and possesses the prospect of further development as an antimicrobial adjuvant therapeutic agent.

Keywords: Compound Huangbai liquid, *S. aureus*, *E. coli,* MRSA

Introduction

Staphylococcus aureus (S. aureus) and *Escherichia coli (E. coli*) are the most common gram-positive and gram-negative pathogens in clinical practice, and have posed a serious threat to human health [1, 2]. *S. aureus* is a major causative agent of a variety of infectious diseases, including breast abscesses, respiratory infections, and sepsis [3-5]. At the same time, *E. coli* is a major causative agent of abdominal abscesses, urinary tract infections, diarrhea, and food poisoning [6-8]. However, with the widespread use and abuse of antibiotics, bacteria such as *S. aureus* and *E. coli* have become increasingly resistant to conventional

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antibiotics. The growing problem of antibiotic resistance and the declining therapeutic efficacy of conventional antimicrobial drugs have become one of the major clinical challenges worldwide, which inevitably increases the risk of treatment failure and disease recurrence [9-12]. Therefore, the development of new antimicrobial strategies, especially alternative or adjunctive therapeutic regimens derived from natural medicines, has become an important direction in the global research of infection therapy [13].

Traditional Chinese medicine is an important natural medicinal resource with thousands of years of history and rich therapeutic experience. Phellodendron bark, as a Chinese herbal medicine, is widely used in traditional Chinese medicine and has good medicinal value. Several studies have confirmed that more than active components (e.g., huangpao alkaloids, flavonoids, flavonoids, etc.) in Phellodendron bark have significant antimicrobial activities and have certain inhibitory effects on *S. aureus* and *E. coli* [14-16]. However, the application of single components is limited to some extent due to the complexity of the pathogen ecosystem and the presence of multiple resistance mechanisms. Therefore, combining Huangbai with other herbal components to form a compound preparation becomes a potential solution. Compound Huangbai Liquid (HB) is a traditional Chinese herbal compound formulation composed of five herbal ingredients: Phellodendron bark, Forsythia fruit, Honeysuckle flower, Dandelion root, and Centipede. Its main active components include cinnamic acid, caffeic acid, quercetin, ursolic acid, and D-limonene, among others. It exhibits significant antibacterial and anti-inflammatory effects [17-19].

HB has been shown to have favorable anti-inflammatory effects in ameliorating inflammation-related diseases such as ulcerative colitis, diabetic foot and atopic dermatitis [20-22]. However, the clinical application of HB in patients with infected abscesses still lacks systematic validation, and there is a gap in research on infected abscesses caused by *S. aureus* and *E. coli* in particular. In this study, we evaluated the clinical efficacy and safety of HB for the first time in two typical abscess populations - abdominal abscess (deep infection) and non-lactating breast abscess (superficial infection) - in patients with *S. aureus* and *E. coli*, and systematically investigated the antimicrobial efficacy and therapeutic potential of HB at multiple levels in conjunction with bacteriological and animal experiments. The results of these studies can provide a scientific basis for further development and application of HB, as well as new therapeutic strategies and approaches for the treatment of infections caused by *S. aureus* and *E. coli*. This is of great significance for alleviating the problem of antibiotic resistance and improving the clinical therapeutic effects.

Materials and methods

Clinical trials-abdominal abscess

Patients: A total of 50 patients with abdominal abscesses who were seen and diagnosed at the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine between January 2018 and June 2023 were retrospectively collected in this study. All patients were clearly diagnosed by clinical and imaging examinations and met the inclusion criteria. The volume of abscess cavity (cm^3) at 3 days postoperatively was used as the main outcome index. Sample size estimation was based on the pretest data (postoperative pus cavity volume of 3.7±1.68 cm^3 in the study group and 5.2±1.68 cm^3 in the control group), and was calculated using the formula for comparing the means of two independent samples. The detailed procedure is described in Supplementary Table 1. It was finally determined that 25 patients were needed in each of the two groups, and the total sample size was 50 cases. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Ethics Approval No. 2020AH-12), and all patients provided written informed consent prior to participation in the study.

Diagnostic criteria: Abdominal abscess [23]: The patient presents clinically with abdominal pain, fever, and localized tenderness. Imaging (Computed Tomography, CT) reveals an accumulation of fluid in the abdominal cavity, and the lesion is usually encapsulated by tissues

such as bowel, visceral organs, omentum, or mesentery, and is clearly separated from the free abdominal cavity. CT suggests the presence of an abscess in the interstitial space of the abdomen or in an abdominal organ and is confirmed by CT-guided puncture to aspirate the pus.

Inclusion and exclusion criteria: Inclusion criteria: ① Meet the diagnostic criteria of abdominal abscess; ② Positive bacterial culture of pus, detecting *S. aureus* or *E. coli*; ③ Age between 18 and 80 years; ④ No serious vital organ disease (such as heart, liver, kidney insufficiency), coagulation dysfunction or immune system disease. Exclusion criteria: ① People with allergy to this drug; ② People who were discharged or transferred to other hospitals in the early postoperative period, which resulted in incomplete follow-up records; ③ People who combined with serious neurological or psychiatric diseases, which affected the compliance with treatment.

Treatment: Control group: ① Basic treatment methods: give fluid rehydration, systemic anti-infection treatment, organ function maintenance and nutritional support. ② Characterization, localization and volume determination of abscess cavity [23]: use ultrasound or CT to determine the formation of its abscess cavity, abscess wall intact (to determine whether there is an indication for flushing), and the volume of abscess cavity, according to the formula: (length × width × height)/2. ③ Placement of drainage tube: put the patient in prone position; provide routine disinfection, lay towels, administrate 2% Lidocaine 5 ml of local anesthesia, place CT-guided puncture needle into the pus cavity. The CT-guided puncture needle enters the pus cavity, extracts the pus, places the guide wire, withdraws the puncture needle, fully expands the skin with the skin expander, introduces the 8F anti-living catheter along the guide wire, and pulls out the core of the needle and the guide wire; the pus is seen to flow out through the catheter, and the drainage bag is connected; disinfect bandage and fix the catheter. ④ Purge the pus cavity: firstly, fully drain out the pus from the pus cavity (usually 12-24 h can drain completely). Determine the initial flushing volume according to the volume of pus cavity; flush the drug of selection gentamicin (2 mL/80000 U, Shanghai Modern Hassan Pharmaceutical Co., Ltd.), each time 8-10 mL, or with 0.9% NaCl injection solution mixed with the ratio of 1:1 flushing. The flushing fluid had a temperature at 38-39°C, and was slowly injected 2 times a day to achieve the disappearance of pus cavity.

Treatment group: ①-③ Same as the control group. ④ Pus cavity irrigation: Use HB (Shandong Hanfang Pharmaceutical Co., Ltd., National Drug Approval Number Z10950097, Batch Number 1308281, 100 mL per bottle) as the irrigation solution. Administer 10-20 mL per session, or mix with 0.9% sodium chloride injection solution in a 1:1 ratio before irrigation. Perform twice daily. For patients with deep abscess locations or those with deep abscesses requiring tube drainage, mix with 0.9% NaCl injection solution in a 1:1 ratio and perform continuous irrigation through the drainage tube for 24 hours. For double-lumen tubes, connect the inflow tube to the mixed solution and the outflow tube to the central negative pressure suction system.

Collection of clinical indicators: Pus samples were collected from patients at the time of puncture and tube placement and on days 7 and 14 after tube placement for bacterial identification and drug susceptibility testing. Blood samples were collected preoperatively and postoperatively on days 3, 7, and 14, respectively, and serum white blood cell (WBC), neutrophil (NEU), C-reactive protein (CRP), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and basic fibroblast growth factor (βFGF) were measured. CT images were used to measure the change in the volume of the abscess cavity before puncture placement and on days 7 and 14 after placement, respectively. The number of days the patients were hospitalized was recorded and they were followed up for one year to observe the recurrence of abdominal abscesses. Adverse effects were also recorded during the treatment and follow-up, including hepatic and renal impairment, skin irritation, diarrhea, drug-related fever and bleeding.

Clinical trials - non-lactating breast abscess abscesses

Patients: A total of 38 patients with non-lactating mastitis who were hospitalized in the

Department of Breast Surgery at the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine between February 2021 and November 2021 were included in this retrospective analysis. All patients were consistent with abscess stage presentation (with or without local skin ulceration) and underwent breast abscess incision and drainage and necrotic tissue removal. The sample size of this study was estimated on the basis of the difference in the overall rate of wound healing between the two groups. Based on the preliminary pre-test data, the total effective rate was set to be 55% in the control group and 85% in the treatment group. Setting $\alpha = 0.05$ (two-sided test) and test efficacy $(1 - \beta) = 0.80$, it was calculated that about 16-17 patients needed to be included in each group, and the total sample size was about 33 cases. The detailed formula and derivation of the sample size estimation are shown in Supplementary Table 1. 38 patients (20 patients in the treatment group and 18 patients in the control group) were included in the study, which was slightly higher than the theoretical estimation, and met the statistical requirements of the preliminary exploratory study. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Ethics Approval No. 2021AH-28), and all patients provided written informed consent prior to participation in the study.

Diagnostic criteria: Breast abscess [24] clinical manifestations are pain and/or lump, fever, chills and discomfort. Ultrasound shows the presence of multiple small abscesses or a single isolated cavity. The abscess is confined or compartmentalized and ultrasound-guided puncture extracts pus.

Inclusion and exclusion criteria: Inclusion criteria: ① Meet the relevant diagnostic criteria of non-lactating mastitis, and clearly diagnosed as periductal chronic mastitis or granulomatous mastitis; ② Non-pregnant or non-lactating, ages older than 18 years and younger than 50 years; ③ No serious diseases of important organs, coagulation system diseases and immune system diseases. Exclusion criteria: ① Allergic to this drug; ② Not in accordance with the prescribed treatment program, or long-term combined use of other drugs with therapeutic effects on non-lactating mastitis, and the efficacy of which cannot be determined; ③ Lack of complete clinical medical records.

Treatment: Control group: ① Basic treatment: all non-lactating mastitis patients complete routine examinations in advance such as blood routine, biochemical series, coagulation routine, immune combination, electrocardiogram, breast ultrasound, breast magnetic resonance and other routine examinations, and exclude contraindications to surgery, preoperative positioning marking, skin preparation, fasting and water for 6 h. Anesthesia adopts general anesthesia, and the surgical procedure is as follows: the patient takes a flat lying position, disinfects and spreads a towel, and then takes the appropriate position to incise the skin and subcutaneous glandular tissue. After incising the skin and subcutaneous glandular tissues in a suitable position, opening the abscess cavity in the breast, releasing the pus, removing all the diseased inflammatory tissues in the breast as far as possible, rinsing with saline and hydrogen peroxide and checking that there is no bleeding point, filling the trauma cavity with Vaseline gauze strips, and fixing the wound by external bandage with sterile gauze, cotton pads, or abdominal bandage. After the operation, the patient is put on oxygen, cardiac monitoring, fasting for 6 h, and temporary drip nutritional support drugs. ② Dressing change from the first postoperative day, only change the external dressing, the gauze block filled in the wound is not taken out, the next day begin to change the dressing in the wound, once a day. Pay attention to the aseptic operation when changing the dressing, keep clean inside the wound, and check whether there is blood seepage, secretion and granulation tissue inside the wound. ③ Start to change the gauze block in the wound on the second day after the operation. When changing the dressing, first use iodophor cotton balls to disinfect and clean the wound around the outer mouth, then gently scrub the wound cavity, and the strength should not be too big, to avoid bleeding. Wipe away the pus and secretion in the wound cavity, and after the wound cavity is clean, fill the wound cavity evenly with sterile gauze block. Use sterile cotton pads to put pressure on the external dressing, and fix it with elastic bandage, adhesive tape or abdominal belt, and change the

dressing once a day for a total of 3 weeks of observation.

Treatment group: ①-③ Same as control group. ④ Purge of pus cavity: patients begin to change the gauze block (Henan Yadu Industry Co., Ltd., production batch number: 2009-CA0095, specification: 80 mm × 80 mm × 8P) in the trauma on the second day after the operation. When changing the medicine, first disinfect and clean the wound around the outer mouth with iodine volts (Shandong Meide Medical Science and Technology Co., Ltd., batch number: 210102, specification: 500 mL per bottle) cotton balls, and then gently scrub the traumatic cavity. The strength should not be too large, in order to avoid bleeding. After that, wipe away the pus and secretion in the trauma cavity. To clean, take the appropriate amount of compound cypress liquid coating (Shandong Hanfang Pharmaceutical Co., Ltd., State Pharmaceutical License: Z10950097, Batch No.: 1308281, specifications: 100 mL per bottle), uniform penetration of gauze block, about 10-20 mL/times, to the extent that gauze block is soaked through but the liquid does not drip out. The gauze block evenly fills the trauma cavity, followed by fixation using external pressure bandage with sterile cotton pads, elastic bandage, tape or abdominal bandage. Change the medicine once a day, and observe for a total of 3 weeks.

Collection of clinical indicators: Cytokines: Measured before treatment and on day 7 of treatment, including levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the budding tissue; Wound healing rate [25]: Measure the size of the wounds on the 7th, 14th, and 21st day after the treatment, and then combine with the area of the original wounds after the operation to calculate the wound healing rate. Specific operation steps: When changing the dressing, use sterile forceps to detect the outermost edge of the wound in all directions, mark the corresponding position on the surface skin, then apply the transparent film evenly to the wound, and draw the area of the wound by connecting the points along the previously marked points. The film was compared with the ECG scanning paper to calculate the specific value, and then the wound healing rate was calculated according to the formula: wound healing rate on day n (%) = (original wound area - measured wound area on day n)/original wound area × 100%; VAS Pain Score [26]: On the 7th, 14th, and 21st days after treatment, the patients were asked to record the self-perceived pain and the pain sensation of the wound during dressing change, the scores ranged from 0 to 10, and the higher score of the VAS meant the stronger the pain sensation. Efficacy evaluation criteria: after 21 days of treatment, the overall efficacy was evaluated according to the healing of the wounds in the two groups. Total Effective = Cured + Significantly Effective + Improved (Supplementary Table 2). Patients were followed up for one year to detect recurrence of breast abscesses; finally, it was recorded whether the patients had any adverse reactions such as bleeding liver and kidney impairment, skin allergy, diarrhea, drug-related fever, and bleeding during the treatment and follow-up period.

Bacterial experiment

Bacterial strains and medication: S. aureus (NCTC8325) and *E. coli* (MPEC6) were supplied by the College of Biological Sciences, Anhui Agricultural University, Anhui Province, China, and the HB was purchased from Maanshan Hospital of Traditional Chinese Medicine, Maanshan City, Anhui Province, China (Specification: 100 mL per bottle; China National Pharmaceutical License No.: Z10950097, Batch No.: 1308281).

Extraction of methicillin-resistant Staphylococcus aureus (MRSA): Initially, samples were aseptically collected from the patient's infection site, such as abscess pus or wound secretions. These samples were then inoculated onto selective media, such as oxacillin-containing mannitol salt agar, for preliminary isolation and incubated aerobically at 37°C for 24-48 hours. Suspected colonies were preliminarily identified through Gram staining and a coagulase test, with methicillin resistance confirmed via the cefoxitin disk diffusion method. Finally, the bacterial strains were preserved in glycerol broth and stored at -80°C for future use.

Growth conditions of the strain and preparation of the bacterial solution: The *S. aureus, E. coli* and MRSA bacterial fluids frozen in the

refrigerator at -80°C were taken out and inoculated onto TSB solid medium for delineation respectively, and the TSB solid medium was placed in overnight culture at 37°C temperature. A pipette gun was used to pick single colonies on the overnight cultured TSB medium. 3 mL of TSB liquid medium was added to the sterilized test tube, and the single colonies dipped on the tip of the pipette gun were blown into the test tube. 37°C, 200 rmp/min was used for overnight culture.

Minimum inhibitory concentration (MIC): The MIC was determined using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Overnight cultures of *S. aureus* NCTC8325, *E. coli* MPEC6, and MRSA clinical isolates were adjusted to an optical density of OD600 = 1.0 (approximately 1×10^8 CFU/mL) and diluted 1:100 in cation-adjusted Mueller-Hinton broth (CAMHB) to achieve a final inoculum of ~5×10^5 CFU/mL. Serial two-fold dilutions of the HB stock solution (0.2 mg/mL) were prepared in CAMHB across a 96-well microtiter plate. Each well was inoculated with 100 µL of the bacterial suspension. The plates were incubated at 35°C for 18-24 hours. The MIC was defined as the lowest concentration of HB that completely inhibited visible bacterial growth. Controls included growth control (bacteria without HB), solvent control (CAMHB with vehicle), and sterility control (CAMHB only). All experiments were performed in triplicate. The MIC values were reported in µg/mL after conversion based on the HB stock concentration.

Bacterial growth experiment: Bacterial suspensions were standardized by measuring the OD600 value to ensure consistent initial inoculum size. Four separate 100 mL LB liquid media were each inoculated with 1 mL of the bacterial suspension. Experimental groups received varying amounts (0.5 mL, 1 mL, 1.5 mL) of HB, while a positive control group was established without the HB. These cultures were incubated at 37°C with shaking at 150 rpm. The OD600 value was recorded every 2 hours until bacterial growth plateaued. This procedure was replicated three times [27].

Determination of colony-forming units (CFU): Within a sterile environment, the overnight bacterial culture was adjusted to OD600 = 1. This was then diluted 1:1000 with TSB liquid medium and mixed thoroughly. Five centrifuge tubes were prepared. The first tube received only 1 mL of the diluted bacterial solution. The subsequent four tubes were supplemented with varying HB doses, resulting in concentrations of 35 µg/mL, 70 µg/mL, 105 µg/mL, and 140 µg/mL for *S. aureus*, and 125 µg/mL, 250 µg/mL, 375 µg/mL, and 500 µg/mL for *E. coli*. Each tube's total volume was adjusted to 1 mL and mixed well. After a 12-hour incubation at 37°C, the solutions underwent a 10-fold serial dilution. Samples from the 10^-5 and 10^-6 dilutions were plated on liquid medium and incubated overnight at 37°C. The number of bacterial colonies on these plates was then counted. This experiment was conducted three times, with three replicates each time [28].

Animal experimentation

Animal modeling and drug administration: BALB/C mice (female, 18-23 g, 7-8 weeks old) were procured from Hangzhou Ziyuan Experimental Animal Technology Co., Ltd. (License No. SCXK (Zhejiang) 2019-0004). All animal procedures were approved by The Experimental Animal Ethics Committee of Anhui University of Traditional Chinese Medicine (Approval No. AHUCM-mouse-2022141) and conducted in accordance with institutional guidelines. A priori sample size calculation was performed based on preliminary data, indicating that n = 15 per group would provide 80% power to detect a 2-log10 reduction in bacterial load with $\alpha = 0.05$. Mice were acclimatized for one week under standard conditions. The mouse full-thickness burn wound infection model was established as previously described. Briefly, mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg). The dorsal hair was removed, and a full-thickness burn was created by applying a copper rod to the exposed skin for 40 seconds. Subsequently, a 20 µL bacterial suspension containing 5×10^7 CFU of *S. aureus* and *E. coli* was topically applied to the burn site. Sham-infected control mice received an equal volume of sterile PBS. Successful infection was confirmed by the presence of an open, purulent wound 48 hours post-inoculation. Post-operative analgesia was provided by administering ibuprofen (30 mg/kg) in drinking water for 48 hours.

Following successful model establishment, mice were randomly assigned to four groups (n = 15 per group per bacterial strain) using a computer-generated random number sequence: (1) Sham-infected Control (PBS treatment), (2) Infected Model (Vehicle control), (3) HB Treatment, and (4) Levofloxacin Treatment. The investigator performing the surgeries and daily observations was blinded to the treatment group allocations during the experiment. Group allocation was concealed until interventions were administered. HB Group: Wounds were topically treated with 20 μL of undiluted Compound Huangbai Liquid (HB, 0.1 g/mL) applied directly to the wound bed, twice daily. Levofloxacin Group: Mice received intraperitoneal injections of levofloxacin (10 mg/kg in saline) once daily. Infected Model Group: Wounds were treated topically with 20 μL of sterile saline, twice daily. Sham-infected Control Group: Wounds were treated topically with 20 μL of sterile PBS, twice daily. Upon completion of the experimental procedures, the animals were euthanized by carbon dioxide inhalation.

Assessment of bacterial burden: On day 7 post-infection, a pre-defined primary endpoint, mice (n = 5 per group) were euthanized. A full-thickness wound tissue sample (approximately 1 cm diameter, weighed) was harvested aseptically. Tissues were homogenized in 1 mL of sterile PBS. Serial 10-fold dilutions were plated on LB agar and incubated at 37°C for 24 h. Bacterial colonies were counted, and the results were expressed as log10 colony-forming units per gram of tissue (log10 CFU/g). Data are presented as scatter plots showing individual animal values as mean ± SD [29].

Histopathological analysis: Mice were anesthetized by intraperitoneal injection of 3% pentobarbital sodium 50 mg kg^{-1}, and were executed by cervical dislocation method, with two mice in each group randomly executed on days 3, 7, and 14 after modeling. Under aseptic conditions, the whole trabecular tissue was cut along the outer edge of the trabeculae of about 2 mm, and the trabecular tissue was fixed, dehydrated, embedded in paraffin, sectioned, hematoxylin stained for 5 min; washed with tap water, 3 times; eosin alcohol treated for 2 min; differentiated, rinsed with 1% ethanol, hydrochloric acid, and cemented [22]. The structural changes of the traumatized tissue at each time point were observed at ×200 magnification. All histopathological slides were coded and evaluated by a pathologist blinded to the treatment groups. A semi-quantitative scoring system was used to assess inflammation (0-4), necrosis (0-3), and granulation tissue formation (0-3).

Enzyme-linked immunosorbent assay (ELISA): Post-anesthesia with 3% pentobarbital sodium (50 mg/kg) administered intraperitoneally, blood samples (3 mL each) were drawn from randomly selected mice on the 3rd, 7th, and 14th days post-modeling (n = 5 per group). Serum concentrations of IL-1, IL-6, TNF-α, VEGF-A, EGF, and TGF-β were determined using enzyme-linked immunosorbent assays (ELISA kits, Keshun Biotechnology, Shanghai, China) [30]. Three sets of wells were prepared: standard, test sample, and blank. Each set received 100 μL of their respective solutions: varying concentrations of standards, test samples, or standard solution diluents. The ELISA plate was sealed and incubated at 37°C for 1 hour. After discarding the liquid and drying the plate, it was rinsed thrice. Subsequently, 50 μL of working solution A was added to each well, followed by a 37°C incubation for 1 hour. The liquid was discarded, the plate dried and washed, and then 50 μL of working solution B was added, following the same procedure. After a 15-minute incubation at 37°C, the reaction was halted by adding 50 μL of stop solution. The optical density (OD) values for each well were recorded using a microplate reader at a wavelength of 450 nm.

Statistical analysis

All statistical evaluations were conducted using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation (mean ± SD). Data from two groups were analyzed using the independent samples t-test. For data from three or more groups, one-way analysis of variance (ANOVA) was employed. If continuous variables did not meet normality or had unequal variances, nonparametric tests were utilized. Count data comparisons were executed using the chi-square test, with a significance threshold set at

Table 1. Comparison of positive bacterial culture results in pus between the two patient groups [n (%)]

Time	Treatment Group n (%)	Control Group n (%)	χ^2/Fisher exact	*P*
7 days after treatment	9 (36%)	16 (64%)	3.92	0.048
14 days after treatment	2 (8%)	4 (16%)	Fisher exact	0.667

Notes: Fisher exact test was used for comparisons with expected counts < 5.

Table 2. Comparison of inflammatory response indicators between the two patient groups (mean ± SD)

Group	Treatment Group (n = 25)	Control Group (n = 25)	*t*/*Z*	*P*
WBC (× 10^9/L)				
Before treatment	13.13±3.06	13.88±2.04	-1.022	0.312
3rd day after treatment	9.07±3.86	12.15±2.51	-3.342	0.002
7 days after treatment	6.70±2.15	8.30±2.28	-2.550	0.014
14 days after treatment	6.38±2.18	5.74±1.16	-1.252	0.211
NEU (× 10^9/L)				
Before treatment	11.04±4.24	11.56±2.37	-.525	0.602
3rd day after treatment	6.77±3.40	8.52±2.49	-2.069	0.044
7 days after treatment	4.94±2.00	6.17±1.25	-2.756	0.006
14 days after treatment	4.58±1.30	4.16±0.90	1.343	0.185
CRP (mg/L)				
Before treatment	13.18±0.87	13.05±0.61	-.699	0.485
3rd day after treatment	13.59±1.45	14.85±2.29	-3.660	0.000
7 days after treatment	6.64±0.66	7.29±0.82	-3.183	0.001
14 days after treatment	6.11±1.54	6.60±1.50	-1.138	0.261

Notes: WBC, white blood cell; NEU, neutrophil; CRP, C-reactive protein.

Table 3. Comparison of tissue growth and healing indicators between the two patient groups (mean ± SD)

Group	Treatment Group (n = 25)	Control Group (n = 25)	*t*/*Z*	*P*
VEGF (pg/ml)				
Before treatment	88.24±6.30	87.27±7.10	.512	.611
3rd day after treatment	145.33±7.64	137.17±7.73	3.750	.000
7 days after treatment	155.80±7.70	147.26±6.78	4.160	.000
14 days after treatment	154.00±9.06	134.59±4.78	-5.559	.000
EGF (pg/ml)				
Before treatment	423.66±14.79	420.57±7.24	.939	.353
3rd day after treatment	523.84±13.93	501.20±11.81	6.195	.000
7 days after treatment	590.57±5.21	535.66±7.41	-6.064	.000
14 days after treatment	615.39±8.28	565.38±7.67	-6.063	.000
βFGF (mg/L)				
Before treatment	3.56±0.74	3.51±0.55	-.466	.641
3rd day after treatment	4.67±0.73	4.20±0.34	2.933	.005
7 days after treatment	5.61±0.70	4.26±0.60	7.344	.000
14 days after treatment	4.98±0.60	4.32±0.38	-3.824	.000

Notes: VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; βFGF, basic fibroblast growth factor.

α = 0.05. $P < 0.05$ was considered statistically significant.

Results

Clinical efficacy of HB in abdominal abscess patients

To evaluate the clinical efficacy and safety of HB in patients with abdominal abscesses, the study involved grouping patients and observing them after HB intervention. No significant differences were observed between the two groups of patients in terms of gender, age, abscess size, comorbidities, and the types and duration of antibiotic use (all $P > 0.05$) (Supplementary Table 3). Following intervention, the bacterial positivity rate in pus samples was significantly lower in the HB group (9/25, 36%) compared to the control group (16/25, 64%) on day 7 post-treatment ($P < 0.05$) (**Table 1**). Serum levels of inflammatory markers (WBC, NEU, CRP) were significantly reduced in the HB group on days 3 and 7 (all $P < 0.05$) (**Table 2**), while levels of growth factors (VEGF, EGF, β-FGF) were significantly elevated on days 3, 7, and 14 (all $P < 0.05$) (**Table 3**). Furthermore, the HB group demonstrated a significantly smaller abscess cavity volume on day 14 ($P < 0.05$) (Supplementary Table 4) and a shorter hospitalization time ($P < 0.05$) (Supplementary Table 5). No significant differences in recurrence rates were observed during follow-up, and no adverse reactions were reported.

Clinical efficacy of HB in non-lactating breast abscess patients

To investigate the therapeutic effects of HB on wound heal-

Table 4. Changes in cytokine levels after treatment in patients with non-lactation mastitis (mean ± SD)

Group	Time	Treatment Group (n = 20)	Control Group (n = 18)	t/Z	*P*
TNF-α (pg/mL)	Before treatment	386.60±75.37	413.59±65.27	-1.174	0.248
	7 days after treatment	360.46±47.46*	404.14±60.47*	-2.490	0.018
IL-1β (pg/mL)	Before treatment	205.26±31.23	222.41±33.41	-1.636	0.111
	7 days after treatment	195.64±21.35*	215.88±36.74*	-2.103	0.043

Notes: TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β. Notes: *$P < 0.05$ Compared with before treatment.

Table 5. Wound healing rate of non-lactation mastitis patients after treatment (mean ± SD)

Group	7 days	14 days	21 days
Treatment group (n = 20)	15.53±1.48	35.66±3.07*	64.91±8.46#
Control group (n = 18)	14.26±1.46	32.30±5.35*	58.43±9.67#
t	2.647	2.404	2.204
p	0.012	0.022	0.034

Notes: *$P < 0.05$ Compared with the 7th day in the same group; #$P < 0.05$ Compared with the 14th day in the same group.

ing, inflammation, and pain relief in non-lactating breast abscess patients, the study further grouped the patients. No significant differences were found in baseline characteristics and types and duration of antibiotic use between the two groups ($P > 0.05$) (Supplementary Table 6). On day 7 post-treatment, levels of local inflammatory cytokines (TNF-α, IL-1β) in wound tissue were significantly lower in the HB group compared to the control group ($P < 0.05$) (**Table 4**). The wound healing rate was significantly higher in the HB group on days 7, 14, and 21 (all $P < 0.05$) (**Table 5**), accompanied by significantly lower VAS pain scores on days 7 and 14 ($P < 0.05$) (Supplementary Table 7). The total effective rate after 21 days was 95.0% in the HB group, significantly higher than the 83.3% in the control group ($P < 0.05$) (**Table 6**). No recurrences were observed in the HB group at the 6-month follow-up, compared to 2 cases (11%) in the control group (Supplementary Table 8), and no adverse reactions occurred.

In vitro antibacterial activity of HB

To validate the in vitro antibacterial effects of HB, the study applied HB interventions to *E. coli*, *S. aureus*, and MRSA isolated from patients, followed by relevant observations. The MIC assessment was conducted utilizing the two-fold dilution technique. The MIC values for HB were determined to be 140 μg/mL for *S. aureus* and 500 μg/mL for *E. coli*. As illustrated in **Figure 1A** and **1B**, bacterial cultures in both the positive control group (devoid of HB) and the group with half the drug concentration exhibited pronounced growth during the lag and logarithmic phases. This growth decelerated in later stages, attributed to nutrient exhaustion. Notably, upon introducing HB at the MIC concentration, a marked suppression of bacterial growth was evident. This inhibitory effect on bacterial proliferation intensified with escalating HB concentrations. Repeated CFU experiments with HB targeting *S. aureus* revealed a discernible impact on CFU counts when HB concentration reached 70 μg/mL, as represented in **Figure 1C**. Analogously, for *E. coli*, repeated CFU experiments highlighted a distinct influence on CFU counts at an HB concentration of 250 μg/mL, as depicted in **Figure 1D**.

To validate the in vitro antibacterial effects of HB, the study applied HB interventions to *E. coli*, *S. aureus*, and MRSA isolated from patients. The MIC was determined using the broth microdilution method, with results showing MIC values of 140 μg/mL for *S. aureus* and 500 μg/mL for *E. coli*. As illustrated in **Figure 1A** and **1B**, bacterial cultures in the positive control (without HB) and the half-MIC concentration group exhibited pronounced growth during the lag and logarithmic phases, which decelerated in later stages due to nutrient exhaustion. Notably, the introduction of HB at the MIC concentration markedly suppressed bacterial growth, with the inhibitory effect intensifying as HB concentrations increased. Repeated colony-forming unit (CFU) experiments demonstrated that HB began to significantly reduce CFU counts of *S. aureus* at a concentration of 70 μg/mL (**Figure 1C**) and of *E. coli* at 250 μg/mL (**Figure 1D**). For the inhibition of MRSA, HB

Table 6. Total postoperative clinical efficacy of non-lactation mastitis patients

Group	Cured	Markedly effective	Improved	Ineffective	Total effective rate (%)
Treatment group (n = 20)	4	12	3	1	95.0
Control group (n = 18)	1	8	6	3	83.3
Z value					2.092
P value					< 0.05

Notes: $P < 0.05$ indicates a statistically significant difference between groups (rank-sum test).

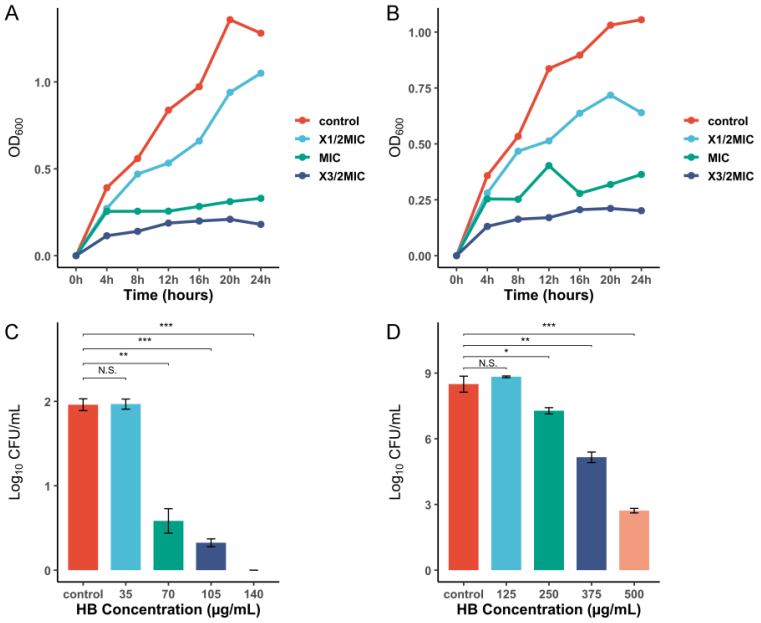

Figure 1. Antibacterial effects of HB. A. The effect of different concentrations of HB on the growth curve of *S. aureus*. B. The effect of different concentrations of HB on the growth curve of *E. coli.* C. The effect of different concentrations of HB on the CFU of *S. aureus*, $*P < 0.05$, $***P < 0.001$; N.S., Not significant. D. The effect of different concentrations of HB on the CFU of *E. coli*, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; N.S., Not significant. HB, Compound Huangbai Liquid. *S. aureus, Staphylococcus aureus. E. coli, Escherichia coli.* CFU, Colony-forming units. MIC, minimal inhibitory concentration.

showed effects similar to those on *E. coli* and *S. aureus* (**Figure 2**).

Efficacy of HB in a mouse wound infection model

On day 7 post-infection, bacterial counts in wound tissues were significantly lower in both the HB and levofloxacin antibiotic groups compared to the model group ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). A significant difference was also observed between the HB and antibiotic groups ($*P < 0.05$) (**Figure 3**). Histopathological examination showed that HB treatment reduced inflammatory cell infiltration and increased fibroblast numbers over time in both *E. coli* and *S. aureus* infected wounds, compared to the model and antibiotic groups (**Figures 4**, **5**). ELISA results indicated that HB treatment significantly reduced serum levels of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and increased levels of tissue growth factors (EGF, VEGF-A, TGF-β) on days 7 and 14 post-infection ($P < 0.05$) (**Figures 6**, **7**).

Discussion

In this study, we systematically evaluated the antimicrobial potential of HB against *S. aureus* and *E. coli* infections through clinical trials, bacteriological experiments and multi-dimensional validation in animal models. The main findings were as follows: (I) clinical trials showed that HB had good clinical efficacy and safety for patients with deep and superficial abscesses; (II) in vitro experiments confirmed that HB possessed good antimicrobial activity against *S. aureus* and *E. coli*; and (III) the results of the mouse animal model demonstrated that HB effectively reduced the bacterial load of the infected wounds of *S. aureus* and *E. coli*, inhibit inflammatory cell infiltration, and significantly promote the tissue repair process. Overall, HB showed good antimicrobial effects at multiple experimental levels, suggesting that it may be an effective supplement to traditional antibiotics and provide a new strategy for the treatment of *S. aureus* and *E. coli* infections.

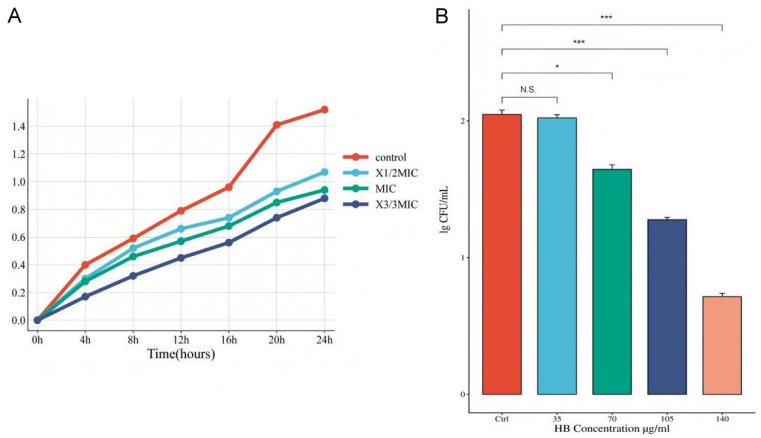

Figure 2. Efficacy of HB against MRSA. A. The effect of different concentrations of HB on the growth curve of MRSA. B. The effect of different concentrations of HB on the CFU of MRSA, *P < 0.05, ***P < 0.001; N.S., Not significant. HB, Compound Huangbai Liquid. *S. aureus, Staphylococcus aureus.* CFU, Colony-forming units. MIC, minimal inhibitory concentration. MRSA, methicillin-resistant Staphylococcus aureus.

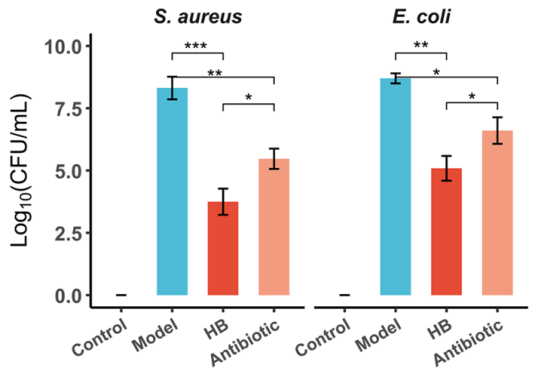

Figure 3. Effect of different treatment groups on *S. aureus* and *E. coli* bacterial culture results. *P < 0.05, **P < 0.01, ***P < 0.001; HB, Compound Huangbai Liquid. *S. aureus, Staphylococcus aureus. E. coli, Escherichia coli.*

Modern pharmacological studies have shown that HB is rich in active compounds such as cinnamic acid, quercetin, ursolic acid and D-limonene, and many of them have been shown to have broad-spectrum antimicrobial effects [17-19]. Zolfaghari et al. [31] reported that cinnamic acid could inhibit the growth of *S. aureus* and *E. coli*, and the mechanism may be through enhancing the permeability of cell membrane, interfering with the key enzyme system of bacteria (e.g. DNA gyrase B), and improving the membrane binding capacity through structural modification of hydrophilic substituents, thus exhibiting good antibacterial activity against *S. aureus* and *E. coli* [32]. Zhou et al. [33] indicated that caffeic acid could exert its effect on the growth of *S. aureus* and *E. coli* by disrupting the cell membrane and ultrastructure of the bacterial cells. Quercetin exhibits antimicrobial activity against *S. aureus* and *E. coli* by disrupting cell membrane structure, inhibiting cell wall synthesis, interfering with DNA and protein synthesis, inhibiting the expression of virulence factors and biofilm formation, and synergizing with antibiotics [34-36]. Ursolic acid inhibits the growth and survival of *S. aureus* and *E. coli* at multiple target levels by disrupting cell wall and membrane structure, inhibiting protein synthesis, inducing reactive oxygen species (ROS) accumulation, promoting lipid peroxidation and DNA rupture, and inhibiting biofilm formation [37, 38]. Meanwhile, D-limonene also showed relevant antimicrobial activities against *E. coli* and *S. aureus* [39], mainly by disrupting the cytoplasmic membrane structure, increasing permeability and inducing leakage of intracellular components, which effectively inhibited the growth of *E. coli* and *S. aureus* [40]. These findings suggest that HB acts synergistically through multi-component and multi-targeting, thus providing a pharmacological basis for its use as an antibiotic alternative or adjunctive therapy.

E. coli is the main causative agent of abdominal abscesses [8], while *S. aureus* is considered a common causative agent in patients with non-lactating breast abscesses [5]. In clinical trials of abdominal abscesses, it was found that the bacterial positivity rate was significantly lower in the treatment group than that in the control group, and serum WBC, NEU and CRP levels were significantly lower, while VEGF, EGF and βFGF levels were significantly higher. In addition, the treatment group had a smaller abscess cavity volume, shorter hospitalization time, and faster overall recovery process. Meanwhile, in the clinical trial of non-lactat-

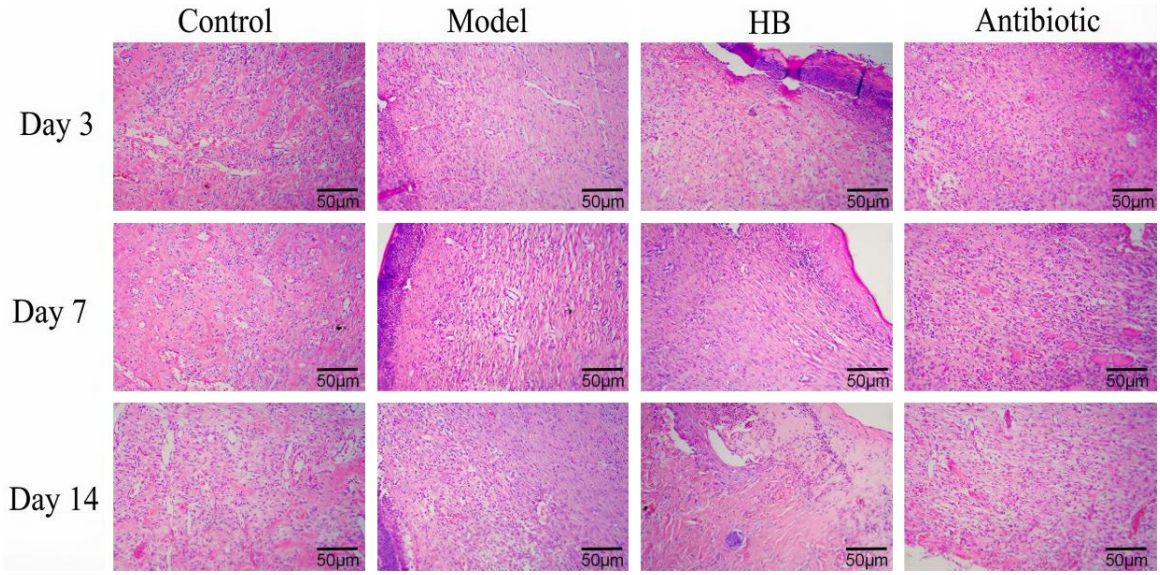

Figure 4. The histopathological features of rat *S. aureus* infected wounds were observed by Hematoxylin and eosin staining (200 magnification). Representative images of each group at different time points were selected. HB, Compound Huangbai Liquid. *S. aureus, Staphylococcus aureus.*

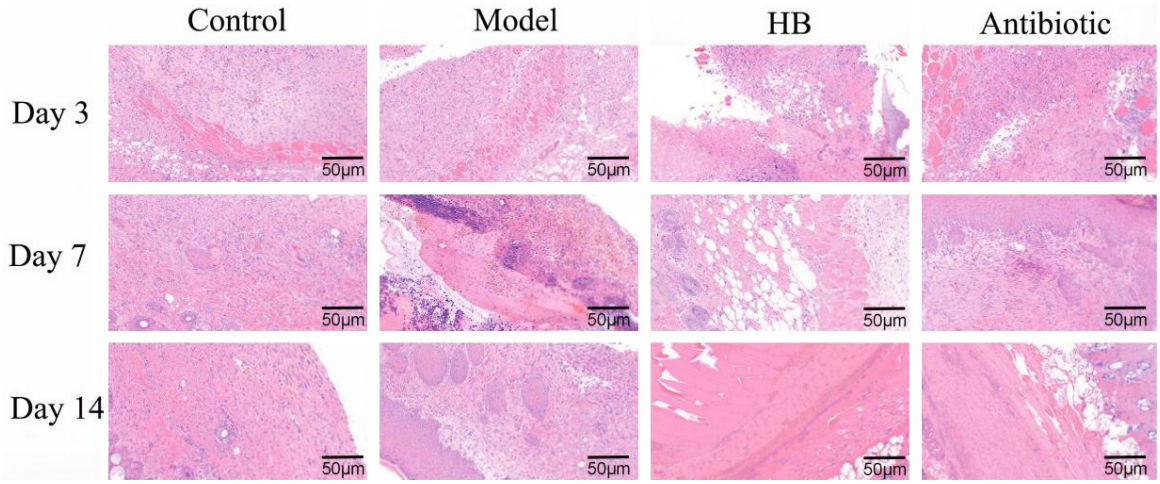

Figure 5. The histopathological features of rat *E. coli* infected wounds were observed by Hematoxylin and eosin staining (200 magnification). Representative images of each group at different time points were selected. HB, Compound Huangbai Liquid. HB, Compound Huangbai Liquid. *E. coli, Escherichia coli.*

ing breast abscess, patients in the treatment group showed a significant decrease in serum TNF-α and IL-1β levels, accelerated wound healing, and lower pain scores than the control group. The clinical efficacy of the treatment group was better than that of the control group, and there was no significant difference in the recurrence rate between the two groups during long-term follow-up. No adverse drug reactions were observed, suggesting that HB has good safety and efficacy. There are fewer clinical cases of HB applied to abdominal abscess and breast abscess. Su et al. [20] applied HB to the treatment of ulcerative colitis, and found that it could significantly reduce the clinical symptoms of patients, reduce the inflammatory reaction, and promote the healing of intestinal mucosa. Yang et al. [41] compared the effect of wet application of compounded Phellodendron Bark liquid with that of antimicrobial calcium alginate wound dressing in the treatment of diabetic foot infection. The results showed that

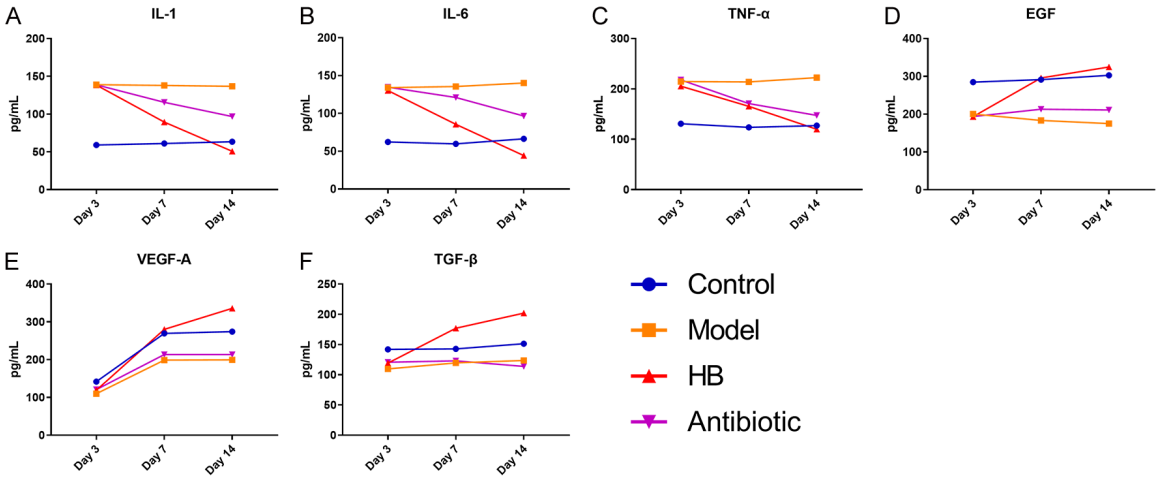

Figure 6. The levels of IL-1 (A), IL-6 (B), TNF-α (C), EGF (D), VEGF-A (E), and TGF-β (F) in the serum of animals with *S. aureus* infected wound models were determined using ELISA. HB, Compound Huangbai Liquid. IL-1, interleukin-1. IL-6, interleukin-6. TNF-α, tumor necrosis factor-α. EGF, epidermal growth factor. VEGF-A, vascular endothelial growth factor-A. TGF-β, transforming growth factor-β.

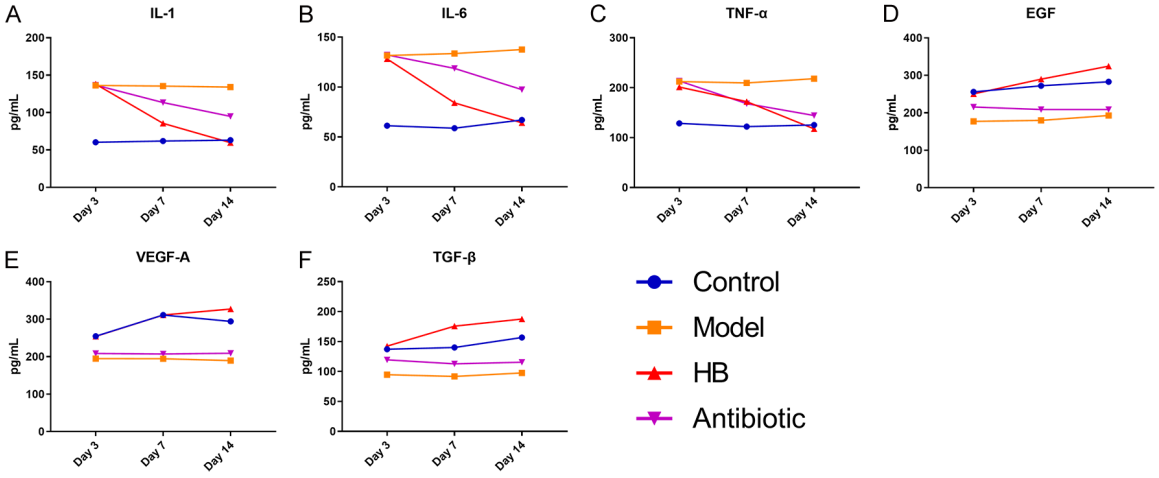

Figure 7. The levels of IL-1 (A), IL-6 (B), TNF-α (C), EGF (D), VEGF-A (E), and TGF-β (F) in the serum of animals with *E. coli* infected wound models were determined using ELISA. HB, Compound Huangbai Liquid. IL-1, interleukin-1. IL-6, interleukin-6. TNF-α, tumor necrosis factor-α. EGF, epidermal growth factor. VEGF-A, vascular endothelial growth factor-A. TGF-β, transforming growth factor-β.

Compound Huangbai Liquid had good efficacy in controlling infection and promoting wound healing, and demonstrated advantages in drug economy. However, it should be noted in clinical operation that the timing of abdominal abscess flushing should be after the formation of the abscess cavity and pus wall, to prevent the drug and pus from overflowing into the abdominal cavity, so that the drug and the pus wall can come into contact to produce effects. At the same time, the amount of flushing should be calculated according to the volume of the pus cavity before flushing, and the dosage of flushing should be changed according to the volume of the pus cavity, to ensure that the drug is sufficient and can be in full contact with the pus cavity.

Currently, antibiotics are the mainstay of treatment for *S. aureus* and *E. coli* infections; however, the widespread use of antibiotics has led to an increase in bacterial resistance, which largely increases the difficulty of treatment [11, 12, 42]. Herbal medicines have been shown to

not only have potent antibacterial activity, but also can effectively avoid the problem of bacterial resistance, providing new means for the treatment of *S. aureus* and *E. coli* infections [43, 44]. The results of our bacterial experiments showed that HB had good antibacterial effects against both *S. aureus* and *E. coli*, and the MICs of HB were 140 μg/mL and 500 μg/mL, respectively, which indicated that HB was able to inhibit the growth of these two pathogens within a certain concentration range. Moreover, the observation of bacterial growth curves at different concentrations of HB showed that the bacteriostatic effect of HB increased with the increase of drug concentration. And the CFU experiment found that the number of *S. aureus* colonies could be inhibited after the concentration of HB reached 70 μg/mL, and the number of *E. coli* colonies could be inhibited after the concentration of HB reached 250 μg/mL. The inhibitory effect was positively correlated with the concentration of HB. The results of CFU experiment also further verified the inhibitory effect of HB. Quercetin, one of the components of HB, can inhibit the growth and reproduction of *S. aureus* and *E. coli* without exerting selective pressure on the bacteria, leading to the occurrence of bacterial resistance [19, 45, 46]. This may be one of the mechanisms of action of HB to inhibit bacteria and avoid bacterial resistance.

The inflammatory environment of infected wounds favors bacterial growth and delays healing of infected wounds. HB has been reported to reduce wound area, symptom score, calcitoninogen, blood sedimentation, and C-reactive protein levels in patients with diabetic foot ulcers, which in turn improves local and systemic inflammation levels and promotes the healing of diabetic foot ulcers [43]. HB also reduces the expression of proinflammatory cytokines, such as IL-1β, IL-4, IFN-γ, IL-13, and IL-17, and increases the expression of anti-inflammatory cytokines, such as IL-10, by decreasing the infiltration of inflammatory cells into inflamed tissues of rats with atopic dermatitis [47]. Our animal experiments also confirmed that HB reduced the bacterial count, inflammatory cell count and serum levels of IL-1, IL-6, and TNF-α in *S. aureus* and *E. coli* infected wounds. In addition, HB increased the number of fibroblasts and serum levels of EGF, VEGF-A, and TGF-β in infected wounds. This indicates that HB not only has the effect of inhibiting the growth of wound bacteria and improving the inflammatory environment, but also has the effect of promoting wound healing and repair.

However, there are some limitations of this study. First, the relatively small sample size and retrospective nature of the clinical trial may have some impact on the reliability and generalizability of the results. Further large-scale prospective clinical trials will be able to more strongly verify the antimicrobial efficacy of HB. Second, the animal experiments in this study can be further deepened, such as the detection of cytokine levels, histological staining and drug resistance mechanism, in order to more comprehensively understand the mechanism of antimicrobial and avoidance of bacterial resistance effects of HB.

In summary, our clinical, bacterial and animal experimental findings revealed that HB possesses antimicrobial effects against *S. aureus* and *E. coli*, and we also found that HB can inhibit inflammation and promote wound healing. It suggests that HB may be a potential antimicrobial drug to provide new therapeutic strategies and methods to improve the treatment efficacy of *S. aureus* and *E. coli* infections and to solve the problem of antibiotic resistance.

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Disclosure of conflict of interest

None.

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References

[1] Sojo-Dorado J, López-Hernández I, Rosso-Fernandez C, Morales IM, Palacios-Baena ZR, Hernández-Torres A, Merino de Lucas E, Escolà-Vergé L, Bereciartua E, García-Vázquez E, Pintado V, Boix-Palop L, Natera-Kindelán C, Sorlí L, Borrell N, Giner-Oncina L, Amador-Prous C, Shaw E, Jover-Saenz A, Molina J, Martínez-Alvarez RM, Dueñas CJ, Calvo-Montes J, Silva JT, Cárdenes MA, Lecuona M, Pomar V, Valiente de Santis L, Yagüe-Guirao G, Lobo-Acosta MA, Merino-Bohórquez V, Pascual A and Rodríguez-Baño J; REIPI-GEIRAS-FOREST group. Effectiveness of fosfomycin for the treatment of multidrug-resistant escherichia coli bacteremic urinary tract infections: a randomized clinical trial. JAMA Netw Open 2022; 5: e2137277.

[2] Tătaru AM, Canciu A, Tertiș M, Cristea C and Cernat A. Staphylococcus aureus - review on potential targets for sensors development. Bioelectrochemistry 2023; 153: 108492.

[3] Ahmad-Mansour N, Loubet P, Pouget C, Dunyach-Remy C, Sotto A, Lavigne JP and Molle V. Staphylococcus aureus Toxins: an update on their pathogenic properties and potential treatments. Toxins (Basel) 2021; 13: 677.

[4] Lam JC and Stokes W. The golden grapes of wrath - staphylococcus aureus bacteremia: a clinical review. Am J Med 2023; 136: 19-26.

[5] Saboo A and Bennett I. Trends in non-lactation breast abscesses in a tertiary hospital setting. ANZ J Surg 2018; 88: 739-744.

[6] Ten Doesschate T, van Mens SP, van Nieuwkoop C, Geerlings SE, Hoepelman AIM and Bonten MJM. Oral fosfomycin versus ciprofloxacin in women with E.coli febrile urinary tract infection, a double-blind placebo-controlled randomized controlled non-inferiority trial (FORECAST). BMC Infect Dis 2018; 18: 626.

[7] Hazen TH, Poonawala H, Saharia KK, Donnenberg MS and Rasko DA. Draft genome sequence of Escherichia coli strain UMD142. Microbiol Resour Announc 2020; 9: e01009-20.

[8] Esposito AC, Zhang Y, Nagarkatti N, Laird WD, Coppersmith NA, Reddy V, Leeds I, Mongiu A, Longo W, Hao RM and Pantel H. Do cultures from percutaneously drained intra-abdominal abscesses change treatment? A retrospective review. Dis Colon Rectum 2023; 66: 451-457.

[9] GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. Lancet 2024; 404: 1199-1226.

[10] Okeke IN, de Kraker MEA, Van Boeckel TP, Kumar CK, Schmitt H, Gales AC, Bertagnolio S, Sharland M and Laxminarayan R. The scope of the antimicrobial resistance challenge. Lancet 2024; 403: 2426-2438.

[11] Willis JA, Cheburkanov V, Chen S, Soares JM, Kassab G, Blanco KC, Bagnato VS, de Figueiredo P and Yakovlev VV. Breaking down antibiotic resistance in methicillin-resistant Staphylococcus aureus: combining antimicrobial photodynamic and antibiotic treatments. Proc Natl Acad Sci U S A 2022; 119: e2208378119.

[12] Kaushik M, Sarkar N, Singh A and Kumar P. Nanomaterials to address the genesis of antibiotic resistance in Escherichia coli. Front Cell Infect Microbiol 2022; 12: 946184.

[13] Álvarez-Martínez FJ, Díaz-Puertas R, Barrajón-Catalán E and Micol V. Plant-derived natural products for the treatment of bacterial infections. Handb Exp Pharmacol 2025; 287: 265-293.

[14] Zhou C, Li J, Tan S and Ye H. Selective extraction of berberine and palmatine from Huangbai powder. Pak J Pharm Sci 2013; 26: 1023-1025.

[15] Asila A, Liu J, Liu J, Li L and Liao J. Immunomodulatory effects of berberine on Staphylococcus aureus-induced septic arthritis through down-regulation of Th17 and Treg signaling pathways. Acta Biochim Pol 2022; 69: 215-226.

[16] Pierpaoli E, Cirioni O, Simonetti O, Orlando F, Giacometti A, Lombardi P and Provinciali M. Potential application of berberine in the treatment of Escherichia coli sepsis. Nat Prod Res 2021; 35: 4779-4784.

[17] Di H, Liu H, Xu S, Yi N and Wei G. Network pharmacology and experimental validation to explore the molecular mechanisms of compound Huangbai liquid for the treatment of acne. Drug Des Devel Ther 2023; 17: 39-53.

[18] Liang J, Huang X and Ma G. Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. RSC Adv 2022; 12: 29197-29213.

[19] Zhang J, Zhou R, Xiang C, Jia Q, Wu H and Yang H. Huangbai liniment accelerated wound healing by activating Nrf2 signaling in diabetes. Oxid Med Cell Longev 2020; 2020: 4951820.

[20] Su S, Wang X, Xi X, Zhu L, Chen Q, Zhang H, Qin Y, Yang B, Che N, Cao H, Zhong W and Wang B. Phellodendrine promotes autophagy by regulating the AMPK/mTOR pathway and treats ulcerative colitis. J Cell Mol Med 2021; 25: 5707-5720.

[21] Zhao Y, Dai X, Sun Y, Liao W, Peng Y and Yang J. Efficacy and clinical value of negative-pressure wound therapy with instillation (NPWTi) of compound Phellodendron liquid in the treatment of diabetic foot ulcer: a prospective co-

hort study. Comput Math Methods Med 2022; 2022: 7540020.
[22] Zheng T, Fan M, Wei Y, Feng J, Zhou P, Sun X, Xue A, Qin CX and Yu D. Huangbai liniment ameliorates skin inflammation in atopic dermatitis. Front Pharmacol 2021; 12: 726035.
[23] Roberts BW. CT-guided intra-abdominal abscess drainage. Radiol Technol 2015; 87: 187CT-203CT; quiz 204CT-187.
[24] Lam E, Chan T and Wiseman SM. Breast abscess: evidence based management recommendations. Expert Rev Anti Infect Ther 2014; 12: 753-762.
[25] Jørgensen LB, Sørensen JA, Jemec GB and Yderstraede KB. Methods to assess area and volume of wounds - a systematic review. Int Wound J 2016; 13: 540-553.
[26] Hu Y, Li Y, Zou J and Xu Y. Indwelling needle puncture and irrigation in the conservative treatment of breast abscess during non-lactation period. J Vis Exp 2023.
[27] Song YJ, Yu HH, Kim YJ, Lee NK and Paik HD. Anti-biofilm activity of grapefruit seed extract against Staphylococcus aureus and Escherichia coli. J Microbiol Biotechnol 2019; 29: 1177-1183.
[28] Qian Y, Xia L, Wei L, Li D and Jiang W. Artesunate inhibits Staphylococcus aureus biofilm formation by reducing alpha-toxin synthesis. Arch Microbiol 2021; 203: 707-717.
[29] Gao K, Zhang B and Zhao F. Antibiofilm effect of curcumin against staphylococcus aureus surface wound biofilm-associated infection: in vitro and in silico. Appl Biochem Biotechnol 2023; 195: 5329-5337.
[30] Uppu DS, Samaddar S, Ghosh C, Paramanandham K, Shome BR and Haldar J. Amide side chain amphiphilic polymers disrupt surface established bacterial bio-films and protect mice from chronic Acinetobacter baumannii infection. Biomaterials 2016; 74: 131-143.
[31] Zolfaghari B, Yazdiniapour Z, Sadeghi M, Akbari M, Troiano R and Lanzotti V. Cinnamic acid derivatives from welsh onion (Allium fistulosum) and their antibacterial and cytotoxic activities. Phytochem Anal 2021; 32: 84-90.
[32] Shan W, Wang Y, Zhang Z, Xing J, Xu J, Xiao W, Shen Y, Guo S and Que H. Qingre Baidu mixture-induced effect of AI-2 on Staphylococcus aureus and Pseudomonas aeruginosa biofilms in chronic and refractory wounds. Exp Ther Med 2019; 17: 3343-3350.
[33] Zhou J, Guo M, Wu D, Shen M, Liu D and Ding T. Synthesis of UiO-66 loaded-caffeic acid and study of its antibacterial mechanism. Food Chem 2023; 402: 134248.
[34] Nguyen TLA and Bhattacharya D. Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules 2022; 27: 2494.
[35] Yang D, Wang T, Long M and Li P. Quercetin: its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longev 2020; 2020: 8825387.
[36] Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, Xiao F, Li Y and Yin W. Bacteriostatic effect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J Food Prot 2018; 81: 68-78.
[37] Liu G, Qin P, Cheng X, Wu L, Zhao W and Gao W. Evaluation of the mechanistic basis for the antibacterial activity of ursolic acid against Staphylococcus aureus. Front Microbiol 2024; 15: 1389242.
[38] Oloyede HOB, Ajiboye HO, Salawu MO and Ajiboye TO. Influence of oxidative stress on the antibacterial activity of betulin, betulinic acid and ursolic acid. Microb Pathog 2017; 111: 338-344.
[39] Costa MDS, Rocha JE, Campina FF, Silva ARP, Da Cruz RP, Pereira RLS, Quintans-Júnior LJ, De Menezes IRA, De S Araújo AA, De Freitas TS, Teixeira AMR and Coutinho HDM. Comparative analysis of the antibacterial and drug-modulatory effect of d-limonene alone and complexed with β-cyclodextrin. Eur J Pharm Sci 2019; 128: 158-161.
[40] Zahi MR, El Hattab M, Liang H and Yuan Q. Enhancing the antimicrobial activity of d-limonene nanoemulsion with the inclusion of ε-polylysine. Food Chem 2017; 221: 18-23.
[41] Yang G, Wang G, Li Z, Deng L, Wang N, Wang X, Zhou T, Zhang J, Lei Y, Wang T, Wang Y, Shao H, Chen M, Zhang K, Zhou M, Wang X, Liu X and Ju S. Efficacy and pharmacoeconomic advantages of Fufang Huangbai Fluid hydropathic compress in diabetic foot infections: a comparative clinical study with antimicrobial calcium alginate wound dressing. Front Pharmacol 2024; 15: 1285946.
[42] Mohammed YHE, Manukumar HM, Rakesh KP, Karthik CS, Mallu P and Qin HL. Vision for medicine: staphylococcus aureus biofilm war and unlocking key's for anti-biofilm drug development. Microb Pathog 2018; 123: 339-347.
[43] Wang L, Huang Y, Yin G, Wang J, Wang P, Chen ZY, Wang T and Ren G. Antimicrobial activities of Asian ginseng, American ginseng, and notoginseng. Phytother Res 2020; 34: 1226-1236.
[44] Rasooly R, Molnar A, Choi HY, Do P, Racicot K and Apostolidis E. In-vitro inhibition of staphylococcal pathogenesis by witch-hazel and green tea extracts. Antibiotics (Basel) 2019; 8: 244.
[45] Qu S, Dai C, Shen Z, Tang Q, Wang H, Zhai B, Zhao L and Hao Z. Mechanism of synergy between tetracycline and quercetin against anti-

biotic resistant Escherichia coli. Front Microbiol 2019; 10: 2536.

[46] Kang X, Ma Q, Wang G, Li N, Mao Y, Wang X, Wang Y and Wang G. Potential mechanisms of quercetin influence the ClfB protein during biofilm formation of staphylococcus aureus. Front Pharmacol 2022; 13: 825489.

[47] Wang Y, Wu X, Chen J, Amin R, Lu M, Bhayana B, Zhao J, Murray CK, Hamblin MR, Hooper DC and Dai T. Antimicrobial blue light inactivation of gram-negative pathogens in biofilms: in vitro and in vivo studies. J Infect Dis 2016; 213: 1380-1387.

Supplementary Table 1. Sample size estimation for the study

Study Condition	Indicator	Formula/Value used	Result/Conclusion
Abdominal abscess	Primary endpoint: cavity size on day 3 (cm^3)	Mean ± SD (treatment: 3.7±1.68; control: 5.2±1.68) Sample size formula: $n = 2 \times ((Z\alpha + Z\beta) \times \sigma/\delta)^2$ Parameters: σ = 1.68, δ = 1.5, Zα = 1.96, Zβ = 1.282 (power = 90%)	$n = 2 \times (3.62976)^2 = 26.34$ Each group requires ~26 patients; actual enrolled: 25 per group (total 50)
Breast abscess	Primary endpoint: total effective rate	Expected rate (control: 55%, treatment: 85%) Sample size formula: $n = ((Z\alpha/2 + Z\beta)^2 \times [P_1(1 - P_1) + P_2(1 - P_2)])/(P_1 - P_2)^2$ Parameters: Zα/2 = 1.96, Zβ = 0.84 (power = 80%)	$n = 2.94/0.09 \approx 32.67$ Each group requires ~16-17 patients; actual enrolled: 20 vs. 18 (total 38)

Supplementary Table 2. Efficacy evaluation criteria for wound healing

Efficacy	Evaluation Criteria
Cured	The wound surface is fully epithelialized and remains closed after one week of observation.
Significantly Effective	Wound area reduced by ≥ 75%, granulation tissue is fresh, and symptoms significantly relieved.
Effective	Wound area reduced by > 25% but < 75%, granulation tissue relatively fresh, and symptoms improved.
Ineffective	Wound area reduced by ≤ 25%, granulation tissue slightly fresh, but minimal growth observed.

Supplementary Table 3. Comparison of clinical data between the two patient groups

Baseline data		Treatment Group (n = 25)	Control Group (n = 25)	t/χ^2	P
Gender	Male	14	10	1.282	0.258
	Female	11	15		
Age (year)		59.72±11.81	54.88±15.32	-0.991	0.322
Abscess size (cm^3)		4.56±1.90	3.93±1.14	1.417	0.164
Combined with diabetes		5	7	0.439	0.508
Types of antibiotic use	β-Lactams	10	12	Fisher exact	1.000
	Aminoglycosides	2	3		
	Fluoroquinolones	13	10		
Duration of antibiotic use (Days)		13.12±2.17	14.20±3.11	1.425	0.161

Supplementary Table 4. Comparison of changes in pus cavity size between the two groups (mean ± SD)

Time	Treatment Group	Control Group	t	P
Before treatment (cm^3)	4.56±1.90	3.93±1.14	1.417	0.163
3rd day after treatment (cm^3)	3.71±1.58*	3.30±1.04*	1.106	0.274
7 days after treatment (cm^3)	2.74±1.13*	2.65±1.00*	0.286	0.776
14 days after treatment (cm^3)	1.43±0.73*	1.94±0.94*	-2.157	0.036

Notes: Compared with the pretreatment of this group *P < 0.05.

Supplementary Table 5. Comparison of hospital days and clinical follow-up between the two groups

	Treatment Group	Control Group	t/χ^2	P
Hospital days (d)	18.8±3.15	21.08±2.74	0.446	0.009
6-month follow-up [n (%)]	1 (4%)	3 (12%)	Fisher exact	0.605
12-month follow-up [n (%)]	0	0	-	-

Notes: Fisher exact test was used for categorical data comparisons due to small sample sizes.

Supplementary Table 6. Baseline characteristics of patients with non-puerperal mastitis

Group		Treatment Group (n = 20)	Control Group (n = 18)	t/Z	P
Age (years)		33.80±6.26	34.00±5.96	-0.101	0.920
Disease course (days)		25.50±9.35	24.83±9.78	0.215	0.831
Postoperative wound area (cm^2)		14.41±1.74	14.02±1.80	0.685	0.498
Types of antibiotic use	β-Lactams	12	11	Fisher exact	1.000
	Aminoglycosides	1	2		
	Fluoroquinolones	7	5		
Duration of antibiotic use (days)		12.87±3.03	13.96±3.86	1.731	0.213

Supplementary Table 7. Comparison of postoperative wound VAS pain scores in non-lactation mastitis patients (mean ± SD)

Group	7 days	14 days	21 days
Treatment group (n = 20)	4.10±1.62	1.55±1.64*	2.05±1.36#
Control group (n = 18)	5.28±1.67	2.94±2.18*	1.78±1.06#
t	-2.204	-2.242	0.684
p	0.034	0.031	0.499

Notes: *P < 0.05 Compared with the 7th day in the same group; #P > 0.05 Compared with the 14th day in the same group.

Supplementary Table 8. Comparison of recurrence rates between the two groups at 6 and 12 months after treatment

Time point	Treatment Group (n = 20)	Control Group (n = 18)	t/χ^2	P
6 months follow-up	0 (0%)	2 (11%)	-	-
12 months follow-up	0 (0%)	0 (0%)	-	-

Notes: Statistical comparison was not performed due to low event rates.