Synergistic effects of baicalein with cefotaxime against *Klebsiella pneumoniae* through inhibiting CTX-M-1 gene expression

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**Abstract**

**Background:** Generation of extended-spectrum β-lactamases is one of the major mechanisms by which clinical *Klebsiella pneumoniae* develop resistance to antibiotics. Combined antibiotics prove to be a relatively effective method of controlling such resistant strains. Some of Chinese herbal active ingredients are known to have synergistic antibacterial effects. This study is aimed to investigate synergistic effects of Chinese herbal active ingredients with cefotaxime on the extended-spectrum β-lactamase positive strains of *Klebsiella pneumoniae*, and to analyze mechanism of synergistic action, providing experimental evidence for clinical application of antimicrobial drugs.

**Results:** For total sixteen strains including fifteen strains of cefotaxime resistant *K. pneumoniae* and one extended-spectrum β-lactamase positive standard strain, the synergy rates of cefotaxime with baicalein, matrine, and clavulanic acid were 56.3 %, 0 %, and 100 %, respectively. The fractional inhibitory concentration index of combined baicalein and cefotaxime was correlated with the percentage decrease of cefotaxime MIC of all the strains ($r = -0.78, p <0.01$). In the group of synergy baicalein and cefotaxime, the transcribed mRNA level of CTX-M-1 after treatment of baicalein was decreased significantly ($p <0.05$). Moreover, the CTX-M-1 mRNA expression percentage inhibition (100 %, 5/5) was significantly higher than non-synergy group (25 %, 1/4) ($p <0.05$).

**Conclusions:** Our study demonstrated that baicalein exhibited synergistic activity when combined with cefotaxime against some of extended-spectrum β-lactamases positive *K. pneumoniae* strains by inhibiting CTX-M-1 mRNA expression. However, no direct bactericidal or bacteriostatic activity was involved in the synergistic action. Baicalein seems to be a promising novel effective synergistic antimicrobial agent.

**Keywords:** Baicalein, Extended-spectrum β-lactamases, *Klebsiella pneumoniae*, Synergistic antibacterial action, CTX-M-1 gene

**Background**

Extended-spectrum β-lactamases (ESBLs) have the ability of hydrolyzing a variety of antibiotics, such as penicillin, cephalosporins, and monobactams. It is the main mechanism for the formation of various kinds of bacterial resistance. ESBL can be suppressed by commonly used β-lactamase inhibitors, such as clavulanic acid by binding to and inhibiting the activity of ESBL when combined with antibiotics. Combined antibiotics prove to be a relatively effective method of controlling such resistant strains [1]. However, in recent years, the emergence of resistant strains of β-lactamase inhibitors results in failure of interactive antibiotic treatment. Seeking for new and effective synergistic antimicrobial agents to overcome bacterial resistance are urgently needed.

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Chinese medical herbs have been a rich resource for the discoveries of alternative synergistic antimicrobial agents. Several studies show that certain active ingredients of Chinese herbs have synergistic inhibitory effects on bacteria with antibiotics, such as baikalein and matrine [2, 3]. Baicalein is a type of flavonoids from the roots of Scutellaria baicalensis and Scutellaria lateriflora, which is one of the most commonly used Chinese herbs in China for the treatment of bacterial infections [4]. Synergies of baikalein were identified in combination with tetracycline or β-lactams against two methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates OM481 and OM584 [2]. Baicalein was also reported to have synergy with gentamicin against vancomycin-resistant *Enterococcus* [5]. Chan et al. reported synergistic effects of baikalein with ciprofloxacin against NorA-over-expressed methicillin-resistant MRSA [6].

*Klebsiella pneumoniae* (*K. pneumoniae*, KP) is a type of Gram-negative bacteria that can cause different types of infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (http://www.cdc.gov/HAI/organisms/klebsiella/klebsiella.html). Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance with a higher detection rate of ESBL [7, 8]. With a wide range of therapeutic benefits, the synergy of baikalein with other antibiotics against *K. pneumoniae* may be identified. The aim of the present study was to investigate antibacterial effects of baikalein in association with cefotaxime against ESBL positive *K. pneumoniae* compared with another candidate Chinese herbal ingredient named matrine, which is a kind of alkaloids containing lactam ring structure from the *Sophora* genus. Moreover, possible mechanisms by which baikalein interacts with cefotaxime against *K. pneumoniae* were studied.

**Methods**

**Reagents and Chinese herbal active compounds**

Cefotaxime was purchased from Harbin Pharmaceutical Group Co., LTD General Pharm Factory. Clavulanic acid and baikalein were purchased from Sigma. Matrine was purchased from National Institutes for Food and Drug Control. Cefotaxime and matrine were dissolved in sterile water. Baicalein was dissolved in dimethyl sulfoxide (DMSO) whose final concentration was less than 1 % according to the Clinical and Laboratory Standards Institute (CLSI, USA). Clavulanic acid was dissolved in Phosphate buffer (pH 6.0, 0.1 mol/L).

**Collection of ESBL positive *K. pneumoniae* clinical isolates and identification**

The clinical isolates of ESBL positive *K. pneumoniae* were collected in the Affiliated Hospital of Harbin Medical University. They were identified using an API20E system (bioM’erieux, Marcy l’Etoile, France) with conventional biochemical methods. Finally 15 strains were randomly selected for this experiment. Quality control strain *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were kept in our laboratory.

**Measurement of β- Lactamase activity of clinical isolates of *K. pneumoniae***

β- Lactamase activity was assessed by nitrocefin test. The ESBL- producing strains were validated according to CLSI recommended methodology [9].

**Determination of the minimum inhibitory concentration (MIC)**

MIC was defined as the lowest concentration of a drug that prevents visible growth of a bacterium. All drugs were diluted in Mueller-Hinton Broth (MHB). Each test well contained bacteria in a final concentration of 5 × 10^5 CFU/mL. After 17 h incubation at 37 °C, they were checked for growth. *Escherichia coli* ATCC 25922 was used as sensitivity control strain. All experiments were repeated three times.

Since baikalein is colorful, we determined to use combined visual observation and spectrophotometer method to identify the MIC of drugs. The OD value of each well was read at 630 nm wavelength. The growth of bacteria after treatment was calculated using formula: bacterial growth rate = 100 × OD_dumb- containing well/OD_dumb- free well, where OD value is obtained by subtracting the background OD value from the measured value in each well. MIC was determined as the lowest concentration of the drug on the inhibition rate of more than 90 % [6].

**Synergy testing of Chinese herbal active compounds with antibiotic using checkerboard dilution method**

To investigate if baikalein and matrine have synergy with cefotaxime against *K. pneumoniae* in vitro, checkerboard dilution method was used [10]. Two drugs were diluted in MHB into 8 gradient concentrations, i.e., 1/32 × MIC- 4 × MIC, each longitudinal column of wells having the same concentration of drug A, and each horizontal row of wells having the same concentration of drug B. The total volume of each well was 200 μL, including 50 μL of drug A, 50 μL of drug B, and 100 μL of bacterial suspension with a final bacterial concentration of 5 × 10^5 CFU/mL. In addition, single drug MIC control wells, drug- free control wells, bacteria- free control wells were established. *Escherichia coli* ATCC 25922 was used as sensitivity control strain. After incubation at 37 °C for 17 h, the MIC value was read. Each experiment was repeated three times. Synergy was determined by calculating the fractional inhibitory concentrations index (FICI) using formula: FICI = MIC_dumb A combined with/MIC_dumb A used alone + MIC_dumb B in combination with/MIC_dumb drug B alone, where MIC_dumb denotes
the MIC of drug A when used in combination, MIC\textsubscript{drug A used alone} denotes the MIC of drug A when used alone, MIC\textsubscript{drug B in combination with} means the MIC of drug B when used in combination, and MIC\textsubscript{drug B alone} means the MIC of drug B when used alone. Based on the FICI, the results of the interactive effects were as follows: FICI ≤ 0.5 means synergy, 0.5 < FICI ≤ 0.75 means partial synergy, 0.76 < FICI ≤ 1 means additive, 1 < FICI ≤ 4 denotes indifferent, FICI > 4 indicates antagonistic [10]. In this study, the synergy and the partial synergy were defined as synergy relationship, while the additive, the indifferent and the antagonistic were classified as non- synergy relationship, in order to facilitate statistical analysis.

Detection of \textit{bla\textsubscript{SHV}}, \textit{bla\textsubscript{TEM}}, \textit{bla\textsubscript{CTX-M-1}}, \textit{bla\textsubscript{CTX-M-9}} in clinical isolates of \textit{K. pneumoniae}

Genomic DNA as templates were prepared using boiling pyrolysis method from clinical isolates of \textit{K. pneumoniae}. Specific PCR primers for genes \textit{bla\textsubscript{SHV}}, \textit{bla\textsubscript{TEM}}, \textit{bla\textsubscript{CTX-M-1}} and \textit{bla\textsubscript{CTX-M-9}} were determined in our previous study [9, 11] listed in Table 1. PCR reaction conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing for 30 s, and extension at 72 °C for 1 min, then extension at 72 °C for 5 min. PCR product was subjected to 1.2 % agarose gel electrophoresis, followed by staining and examination.

Measurement of mRNA transcriptional expression levels of \textit{bla\textsubscript{TEM}}, \textit{bla\textsubscript{CTX-M-1}} and \textit{bla\textsubscript{CTX-M-9}} in the clinical isolates of \textit{K. pneumoniae} by reverse transcription (RT)-PCR

Total RNAs were isolated using TRIzol (Invitrogen, Carlsbad, CA) method [9] from the bacteria. Random primers (Takara) and Moloney murine leukaemia virus reverse transcriptase (Promega) were used for RT, then PCR was run using bacterial 16SrRNA as internal control, primers 5' -GGA CGG GTG AGT AAT GTC- 3' and 5' -ACA CCT GGA ATT CTA CCC- 3'. The expected amplified fragment was 578 bp, and the annealing temperature was 56 °C. The primers and other reaction conditions were the same as in Table 1. The product was subjected to 1.2 % agarose gel electrophoresis. Then it was stained and analysis of target band was performed using grayscale analysis software Image J to generate relative mRNA expression levels. The intensity was expressed as a value relative to that of the 16SrRNA [12]. Each experiment was repeated three times.

Counting of viable \textit{K. pneumoniae} after treatment of baikalein and measurement of transcriptional expression of ESBL genes

To further understand the mechanisms by which baikalein works in combination with cefotaxime against these clinical isolates, we repeated the experiments with baikalein alone at the lowest inhibitory concentration determined during combination. Baicalein was added to the same MHB with clinical strains at the lowest inhibitory concentration determined when used in combination with cefotaxime. Bacterial concentration was 5 × 10^5 CFU/mL. Blank control without baikalein was used for comparison. After 17 h incubation at 37 °C, 50 μL of bacterial suspension was taken for serial 10-fold dilution. Approximately 10 μL of bacterial inoculum was inoculated on the medium of agar plates for 17 h at 37 °C. Then viable bacterial counting was conducted. All tests were performed in triplicate. The results were expressed as mean ± standard deviation using CFU/mL as unit. At the same time, the mixed baikalein and bacterial inoculum was used for total RNA isolation. RT-PCR performed in the same ways as above. Each experiment was repeated three times.

Statistical analysis

Statistical analysis was performed using the Fisher’s Exact Test, Student’s t test and correlation analysis with SPSS 16.0 software. \(p < 0.05\) was considered statistically significant.

Results

Interactive antibacterial effects of Chinese herbal active ingredients and clavulanic acid with cefotaxime

To investigate if baikalein can interact with cefotaxime in the control of \textit{K. pneumoniae}, synergy testing was

| Primer | Sequence(5' → 3') | Nucleotide position | Tm | Genbank accession No. | Size |
|--------|------------------|---------------------|----|------------------------|------|
| SHV-F  | TCTCCCTGTAGGACACCCTG | 224-243             | 59 °C | AF124984               | 593 bp |
| SHV-R  | CACACGCACGCACGCGGT | 797-816             |       |                        |      |
| TEM-F  | GTATCCGCTCATGAGACAATA | 154-174            | 56 °C | AB194682               | 717 bp |
| TEM-R  | AGAATGGTGCCTGCAACTT | 851-870             |       |                        |      |
| CTX-M1-F | CCGTGTGCGAGTTGCGAC | 264-280            | 56 °C | X92506                 | 551 bp |
| CTX-M1-R | ACACGGATATCATCTGCCGTT | 798-814          |       |                        |      |
| CTX-M9-F | ATGGGAATAGGAGAGAGAGCA | 132-151           | 56 °C | AJ416345               | 868 bp |
| CTX-M9-R | CCGTGTGCGAGTTGCGAC | 983-1000           |       |                        |      |
conducted on baicalein, matrine, and clavulanic acid with cefotaxime using checkerboard dilution method. The results (Table 2, Fig. 1a) showed that when combined with cefotaxime, baicalein exhibited synergistic effects on some antibiotic-resistant ESBL-positive strains of \( K. \) pneumoniae (56.3 %). But no synergy was observed with matrine (0 %). On the contrast, the positive control drug clavulanate acid showed 100 % synergetic. These findings indicated that baicalein may have moderate synergy with cefotaxime against \( K. \) pneumoniae in vitro. A further correlation analysis demonstrated that the FICI of baicalein and cefotaxime was negatively correlated with the percentage of cefotaxime MIC decrease \((r = -0.78, p < 0.01)\) (Fig. 1b).

### Number of \( K. \) pneumoniae after baicalein treatment in interactive concentrations

To further investigate if baicalein can directly inhibit bacterial growth independently, the strains of clinical ESBL positive \( K. \) pneumoniae in synergy group was treated alone with baicalein at the same lowest inhibitory concentration determined when used in combination with cefotaxime. Each strain was treated both by baicalein alone and no baicalein. After incubation, counting of viable bacteria was conducted. The viable bacterial counting revealed that there was no significant difference \((P > 0.05)\) in the number of viable bacterial colonies between baicalein treated and blank control groups (Fig. 2). This finding suggests that baicalein may not have direct bactericidal action when used in combination with cefotaxime against \( K. \) pneumoniae.

### Distribution of ESBL genes and their mRNA expression changes in \( K. \) pneumoniae treated with interactive concentration baicalein

To investigate if the synergy of baicalein with cefotaxime is associated with the distribution of resistant genes in the clinical strains of \( K. \) pneumoniae, the percentages of ESBL resistant genes, including \( bla_{SHV} \), \( bla_{TEM} \), \( bla_{CTX-M-1} \), \( bla_{CTX-M-9} \) were compared between synergy group and non-synergy group (Fig. 3). The results showed that there were 2 strains with \( bla_{SHV} \) in the synergy group; 12 strains with \( bla_{TEM} \) both in synergy group and non-synergy group (each \( n = 6 \)). The percentage of \( bla_{TEM} \) was 75 % in the synergy group and 85.7 % in non-synergy group. There were 9 strains with \( bla_{CTX-M-9} \), including 5 strains in synergy group with 62.5 % and 4 strains in non-synergy group with 57.1 %. There were 9 strains with \( bla_{CTX-M-9} \), including 5 strains in the synergy group with 62.5 % and 4 strains in the non-synergy group with 57.1 %. Comparison analysis showed that there was no significant difference in the distribution of the four common ESBL resistance genes \((P > 0.05)\), suggesting that the synergy of baicalein and cefotaxime may not be associated with the distribution of these resistance genes.

To further investigate if baicalein interacts with cefotaxime through regulation of gene expression, 15 clinical strains of ESBL positive \( K. \) pneumoniae were treated with baicalein alone at the same MIC determined during

### Table 2 Interactive effects of Chinese herbal active ingredients with cefotaxime on antibiotic resistant \( K. \) pneumoniae

| Strains No. | \( \text{MIC}_{\text{alone}} (\mu \text{g/mL}) \) | \( \text{MIC}_{\text{combined}} (\mu \text{g/mL}) \) | FICI | \( \text{MIC}_{\text{combined}} (\mu \text{g/mL}) \) | FICI | \( \text{MIC}_{\text{combined}} (\mu \text{g/mL}) \) | FICI |
|-------------|----------------------------------|----------------------------------|------|----------------------------------|------|----------------------------------|------|
| 28          | >256  >256  16  128              | 128  128                          | 1.5  2  128                        | 1.008 0.5  4 | 0.063*                         |
| 30          | >256  >256  8  128               | 64  64                            | 0.75 2  128                        | 1.008 0.5  4 | 0.094*                         |
| 58          | >256  >256  32  256              | 128  128                          | 1  2  256                         | 1.008 0.5  8 | 0.047*                         |
| 64          | >256  >256  32  256              | 1  256                           | 1.004 2  256                      | 1.008 0.5  8 | 0.047*                         |
| 80          | >256  >256  32  512              | 64  256                           | 0.75 2  1024                      | 2.008 0.5  16 | 0.047*                         |
| 90          | >256  >256  8  1024             | 128  1024                         | 1.5 2  1024                       | 1.008 0.5  32 | 0.094*                         |
| 102         | >256  >256  16  128             | 32  64                           | 0.63 2  128                       | 1.008 0.5  4 | 0.063*                         |
| 116         | >256  >256  8  128              | 128  64                           | 1  2  128                        | 1.008 0.5  4 | 0.063*                         |
| 171         | >256  >256  16  128             | 128  64                           | 1  2  128                        | 1.008 0.5  4 | 0.063*                         |
| 179         | >256  >256  8  1024             | 64  256                           | 0.5 2  1024                       | 1.008 0.5  32 | 0.094*                         |
| 210         | >256  >256  8  256              | 128  64                           | 0.75 2  256                       | 1.008 0.5  8 | 0.094*                         |
| 219         | >256  >256  8  1024             | 1  1024                          | 1.004 2  1024                     | 1.008 0.5  32 | 0.094*                         |
| 796         | >256  >256  8  256              | 32  64                           | 0.38 2  256                       | 1.008 0.5  8 | 0.094*                         |
| 826         | >256  >256  8  1024             | 128  256                          | 0.75 2  1024                      | 1.008 0.5  32 | 0.094*                         |
| 863         | >256  >256  8  256              | 128  64                           | 0.75 2  256                       | 1.008 0.5  8 | 0.094*                         |
| 700603      | >256  >256  8  4                | 4  2                             | 0.52 2  4                         | 1.010 1  1 | 0.38*                         |

*FICI* ≤0.75 means synergy group including both synergy and partial synergy
*FICI* <0.5 synergy, 0.5 <*FICI* ≤0.75 partial synergy, 0.76 <*FICI* ≤1 additive, 1 <*FICI* ≤4 indifferent, *FICI* >4 antagonistic

*Baica: Baicalein; Mat: Matrine; Cla: Clavulanate Acid*
synergy testing. After incubation, the effect of baicalein on mRNA expression of these resistance genes was studied using RT-PCR. The results showed that baicalein significantly inhibited the expression of CTX-M-1 in strains KP30, KP80, KP179, KP796, KP826, KP219 (P < 0.05) (Figs. 4, 5, and 6, Table 3). Moreover, the CTX-M-1 mRNA expression percentage inhibition (100 %, 5/5) was significantly higher than non-synergy group (25 %, 1/4) (p < 0.05), implying that synergy of baicalein with cefotaxime may be associated with the inhibition of CTX-M-1 mRNA expression.

Discussion

ESBLs play a major role in the development of antibiotic resistance in Gram- negative bacteria. It can damage the structure of β-lactam antibiotics, preventing their binding to penicillin binding protein. ESBL encoding genes consist mainly of SHV, TEM, CTX-M, OXA, GES, PER, and VEB [13]. The most common ESBL genes in K. pneumoniae are SHV, TEM, and CTX-M [14], among them CTX-M being the dominant gene for β-lactam antibiotic resistance in ESBL positive K. pneumoniae [15, 16]. Based on their amino acid changes, CTX-M type of β-lactamases are mainly divided into five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. CTX-M-14 (belonging to CTX-M-9 group) and CTX-M-15 (belonging to CTX-M-1 group) are two major genes in mainland China [17]. For example, a recent study identified 88 % of CTX-M-1 ESBLs among 92 CTX-M ESBL-positive strains of K. pneumoniae isolated from respiratory tract samples [18]. Therefore, the four

![Fig. 1](image1.png)

Fig. 1 Synergy comparison and correlation analysis of FICI with cefotaxime MIC value decrease. (The synergy testing of baicalein, matrine, and clavulanic acid with cefotaxime in bacterial inhibition showed that different synergy rates, which is the percentage of synergistic strains among the total strains studied, were observed with cefotaxime (a). Correlation between the FICI of baicalein with cefotaxime and baicalein-induced cefotaxime MIC decrease percentage was analyzed using SPSS (b). X-axis denotes the FICI of baicalein with cefotaxime, Y-axis means cefotaxime MIC decrease percentage)

![Fig. 2](image2.png)

Fig. 2 Effects of interactive concentration baicalein on the growth of K. pneumoniae. (For each strain, Bai (+) and Bai (−) were compared. Bai (+) denotes baicalein treated strain; Bai (−) denotes blank control strain. Each experiment was conducted in triplicate. X-axis denotes bacterial strain ID; Y-axis means log10 value of bacterial numbers)

![Fig. 3](image3.png)

Fig. 3 Comparison of ESBL gene percentage among different groups. (The percentage of four common ESBL resistance genes in the synergy group and non-synergy group was compared using Fisher’s Exact Test with SPSS software, p < 0.05 was considered statistically significant. Black columns represent the percentage of the target genes in the synergy group, while white columns denote the percentage of the target genes in the non-synergy group)
Fig. 4 Effect of baicalein on the mRNA expression of TEM. (Bai (+) denotes baicalein treated strain in black columns; Bai (-) means blank control strain in white columns. Each strain was divided into baicalein treated and blank control subgroups for comparison of the effect of baicalein on mRNA expression. RT-PCR products were analyzed using Image J software. The mRNA level was expressed as the gray value of target gene relative to that of the 16SrRNA. Each experiment was done in triplicate. The mRNA value was expressed as mean ± standard deviation. The difference was analyzed using Student’s t test. *p < 0.05 meaning statistically significant.

Fig. 5 Effect of baicalein on the mRNA expression of CTX-M-1. (same as Fig. 4 in explanation)
commonly seen genes in mainland China, including SHV, TEM, CTX-M-1, and CTX-M-9, were selected as target resistance genes in this study.

Clavulanic acid as a commonly used β-lactamase inhibitor in practice can competitively bind with β-lactamases, forming acyl-enzyme complex to inhibit their activities, thereby cooperating with antibiotics. But clavulanic acid resistant clinical strains [19] have occurred.

Chinese herbal active ingredients, including mainly flavonoids and alkaloids, have antibacterial activity and less toxicity. Baicalein is isolated from Chinese herb as flavonoid, which has synergistic antimicrobial effects [5, 6, 20]. This study demonstrated that baicalein may cooperate with cefotaxime to inhibit ESBL positive K. pneumoniae. But baicalein can only partially inhibit resistant strains of ESBL positive bacteria through suppressing the mRNA expression of CTX-M-1. Meanwhile, there was no remarkable change in the number of viable bacteria when treated alone with baicalein, implying that baicalein exhibits synergistic antibacterial effect through non-bacteriostatic nor bactericidal mechanisms.

Our previous studies showed that there was difference in the mRNA expression level of ESBL resistance gene SHV in clinical strains of K. pneumoniae. The variation was also associated with antibiotic resistance in bacteria. Therefore we proposed a new strategy for managing bacterial resistance through regulating the expression of ESBL resistance genes [9]. However, there is no report on whether some medicine may have antibacterial effects by inhibiting the expression of resistant genes.

In this study, we first validated the synergy of baicalein with cefotaxime. Then we ruled out the possibility of bacteriostatic or bactericidal activities of synergistic baicalein. The effects of difference in resistance gene distribution on antibiotic resistance in bacterial strains were also investigated. This is the first report on interaction mechanism by which baicalein works with antibiotics through regulating the expression of resistance genes.

Relevant studies and our work showed that bacterial CTX-M gene is associated with cefotaxime resistance [11, 21]. CTX-M gene transfer experiments also confirmed that the CTX-M gene enables the bacteria to cefotaxime

| Table 3 Relationship of combined baicalein with cefotaxime and the mRNA level of resistant genes |
|-----------------------------------------------|------------------------|------------------------|------------------------|------------------------|
| Group                         | Synergy | Non- synergy | P value |
|--------------------------------|---------|--------------|---------|
| TEM mRNA                      | 100(6/6) | 0(0/6) | 33.3(2/6) | 66.7(4/6) | 0.061 |
| CTX-M-1 mRNA                  | 100(5/5) | 0(0/5) | 25(1/4) | 75(3/4) | 0.048* |
| CTX-M-9 mRNA                  | 100(5/5) | 0(0/5) | 75(3/4) | 25(1/4) | 0.444 |

Based on the information in the Figs. 4, 5 and 6, the percentage of inhibited strains for each gene in synergy group and non-synergy group was compared using Fisher's Exact Test with SPSS
* p <0.05 statistically significant
resistance [22]. In this study, down regulation of CTX-M-1 gene expression was found to be associated with cefotaxime MIC decrease. However, genes TEM and CTX-M-9 were not determinants of K. pneumoniae resistance to cefotaxime. It was shown that the gene expression of TEM, CTX-M-1, and CTX-M-9 was inhibited by baicalein in a clinical strain of bacteria, K. pneumoniae 219. But no synergy and cefotaxime MIC value decrease were observed. The possible reasons for this may be that this strain has various types of β-lactamase genes or other resistance mechanisms which cover up the inhibitory effect of baicalein on the expression of certain ESBL genes.

In summary, the present study investigated the interactive effect of baicalein on bacterial drug resistance at molecular level. Our findings may pave a new way for further searching for synergistic antimicrobial drugs. More work should be done to confirm how baicalein down-regulates gene expression and why it only works in some strains.

Conclusions
Our results demonstrated that baicalein exhibited synergistic activity when combined with cefotaxime against some of ESBL positive K. pneumoniae strains by inhibiting CTX-M-1 mRNA expression. However, no direct bactericidal or bacteriostatic activity was involved in the synergistic action. Baicalein seems to be a promising novel effective synergistic antimicrobial agent.

Availability of data and materials
The data supporting the conclusions of this article are included within the article and in Additional file 1. All accession numbers for assayed genes can be found in Table 1.

Authors’ contributions
WHC: Conceived and designed the experiments; Performed the experiments, Analyzed the data. YMF: Conceived and designed the experiments, Contributed reagents/materials/analysis tools, Analyzed the data. FMZ: Conceived and designed the experiments; Analyzed the data. Reviewed the initial and final drafts of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable. Since this is a retrospective analysis of clinical samples, no consent to participate was requested from donors.

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Additional file

Additional file 1: Table S1-1 for Fig. 2. Effects of interactive concentration baicalein on the growth of K. pneumoniae(Log10(CFU/ml)). Table S1-2 for Fig. 3. Comparison of ESBL gene percentage among different groups. Table S1-3 for Fig. 4. Effect of baicalein on the mRNA expression of TEM. Table S1-4 for Fig. 5. Effect of baicalein on the mRNA expression of CTX-M-1. Table S1-5 for Fig. 6. Effect of baicalein on the mRNA expression of CTX-M-9. (XLS 22 kb)

Abbreviations
Bai, baicalein; Cla, clavulanate acid; CLSI, Clinical and Laboratory Standards Institute; DMSO, dimethyl sulfoxide; ESBL, extended-spectrum β-lactamase; ESBLs, extended-spectrum β-lactamases; FICI, fractional inhibitory concentrations index; KP, K. pneumoniae; Klebsiella pneumoniae; Mat, matrine; MHB, Mueller–Hinton Broth; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; PCR, polymerase chain reaction; RT, reverse transcription

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