Baicalin inhibits *Escherichia coli* isolates in bovine mastitic milk and reduces antimicrobial resistance

Q. Y. Zhao, F. W. Yuan, T. Liang, X. C. Liang, Y. R. Luo, M. Jiang, S. Z. Qing,¹ and W. M. Zhang¹
College of Veterinary Medicine, Northwest A&F University, Yangling 712100, Shaanxi, China

ABSTRACT

In this study, we aimed to evaluate the inhibitory effect of baicalin on *Escherichia coli* in vitro and the effects of baicalin treatment on antimicrobial resistance of the *E. coli* isolates. Through isolation, purification, and identification, a total of 56 *E. coli* strains were isolated from 341 mastitic milk samples. The study of inhibition effect of baicalin on the *E. coli* strains in vitro was focused on permeability and morphology of the isolates using an alkaline phosphatase kit and scanning electron microscopy. Furthermore, the resistance spectrum of the isolates to the common antimicrobial agents was tested at sub-minimum inhibitory concentrations of baicalin by the agar dilution method. Extended-spectrum β -lactamase and plasmid-mediated quinolone resistance genes were amplified by PCR before and after incubation with baicalin. The results revealed that baicalin has certain inhibitory effects on the isolates in vitro. The alkaline phosphatase enzyme activity was significantly increased from 1.246 to 2.377 U/100 mL, and the surface of *E. coli* was concave and shriveled. Analysis of the resistance spectrum and PCR amplification showed that, after administration with baicalin, the sensitivity of most strains to the selected antimicrobial agents was enhanced. Strikingly, the drug-resistant genes from 71.43% (40/56) of these isolates were found to have drug-resistant genes to different extents. Altogether, the current study confirmed both the inhibitory effect on *Escherichia coli* in vitro and the reduction of antimicrobial resistance by baicalin. This is the first comprehensive study to report on baicalin, a traditional Chinese medicine that acts on *E. coli* isolated from the mastitic milk samples.

Key words: baicalin, *Escherichia coli*, multi-drug resistance, bovine mastitis

INTRODUCTION

Bovine mastitis, inflammation of the mammary gland, is a damaging and costly disease facing dairy farmers throughout the world (Halasa et al., 2007; Heikkilä et al., 2012). Due to the large-scale adoption of the 5-point plan, mastitis has been successfully controlled in many countries for decades (Neave et al., 1969; Hillerton et al., 1995; Zadoks et al., 2002), although the incidence of pathogens, such as *Escherichia coli* (Olde Riekerink et al., 2010), *Staphylococcus aureus* (Olde Riekerink et al., 2008), and *Streptococcus uberis* (Levison et al., 2016), is still prevalent on many farms. As a major pathogen causing environmental mastitis, *E. coli* is difficult to be cured because of its ubiquitous existence, easy transmission, and rapidly emerging drug resistance properties (Olde Riekerink et al., 2010; Steeneveld et al., 2011; Saini et al., 2012; Dahmen et al., 2013).

For successful implementation of a clinical *E. coli* mastitis control program, it is important to identify the pathogen. This microbiological analysis can be done through isolation and identification of pathogenic bacteria from infected cows. Antimicrobial agents are used for therapeutic as well as preventive measures against bacterial infections including bovine mastitis in farm animals (Lindeman et al., 2013). Unfortunately, several recent studies have reported the increasing occurrence of highly multi-drug-resistant *E. coli* isolated from food-producing animals from various countries including China (Rao et al., 2014; Xu et al., 2015; Seni et al., 2016). Therefore, the need for a safe, novel, cost-effective, alternative therapeutic strategy, especially of herbal origin, is extremely urgent. Susceptibility of bacteria to antimicrobial agents decreases with increasing resistance. Because mechanisms of bacterial resistance are numerous and intractable, reducing or eliminating antimicrobial resistance is challenging.

Traditional Chinese medicines have long been used to treat a wide range of pathologies, and research about their mechanisms of pharmacological activity is very extensive. Some reports have shown that *Scutellaria baicalensis*, one of the most popular and multi-purpose herbs, has strong antimicrobial effects in vitro. Related

Received June 19, 2017.

Accepted October 11, 2017.

¹Corresponding authors: suzhuqing@163.com and ylzhangwm@163.com

research has reported that *S. baicalensis* contains numerous flavonoids, such as baicalin, baicalein, wogonin, and chrysin (Li and Chen, 2005). Some of these flavonoids such as baicalin have been demonstrated to have antioxidant (Chan et al., 2008), antibacterial (Huang, 1999), antiviral (Huang et al., 2000), and antiinflammatory activities (Huang et al., 2006). In addition to its multi-purpose pharmacological actions, baicalin's bacteriostatic mechanisms include destruction of bacterial cell membranes; inhibition of bacterial DNA, RNA, and protein biosynthesis; and degradation of endotoxins (Wu, 2011). However, no research has evaluated the effects of baicalin on *E. coli* isolated from mastitic milks. Therefore, in the present study, we isolated *E. coli* from mastitic milk samples and investigated the antibacterial activity of baicalin against *E. coli* in vitro and its effect on drug resistance.

MATERIALS AND METHODS

Materials

Baicalin (purity >95%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Antimicrobial pretreated paper discs and biochemistry reaction tubes were purchased from Hangzhou Tianhe Microorganism Reagent Co. Ltd. (China). Mueller-Hinton, MacConkey (MAC), eosin methylene blue agar, Luria-Bertani (LB), and Mueller-Hinton broth medium were purchased from Beijing Aoboxing Bio-Tech Co. Ltd. (China). Taq DNA polymerase was purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (China). An alkaline phosphatase (AKP) kit was purchased from Nanjing Jiancheng Bioengineering Institute (China). All other chemicals were of reagent grade.

Bacteriological Examination

Milk samples of mastitic cows ($n = 341$) were collected from 26 dairy herds located in 4 cities (Baoji, Weinan, Xi'an, and Yangling) of Shaanxi province of China. Milk samples were collected using procedures outlined by Rajala-Schultz et al. (2004). Briefly, dirt, bedding, and hair were brushed from teats using a dry paper towel. Teats were then dipped in 4% sodium hypochlorite. Germicide was allowed to contact teats for at least 30 s before being thoroughly dried with single-service paper towels. Teat ends were scrubbed with cotton balls moistened with 70% ethyl alcohol. The 10-mL milk samples collected from each mammary quarter at the front end were discarded. Five to 10 mL of milk from each mammary quarter was collected

into a separate sterile polypropylene test tube. Samples were frozen and transported immediately to the laboratory for microbiological culture.

The milk samples were inoculated in LB broth and incubated for 12 to 18 h at 37°C. Then, the incubated samples were streaked on a MAC plate for 18 to 24 h at 37°C. With a sterile loop, 1 or 2 pink colonies from the MAC plate were picked up and streaked on the other MAC plate for 18 to 24 h at 37°C. Further identification of *E. coli* was performed according to the methods described by the National Mastitis Council (Barnes Pallesen et al., 1987). Routine bacterial examination, such as colony morphology, Gram stain test, typical growth on eosin methylene blue agar (Hogan et al., 1999), and micro-biochemical reaction were performed. The specific 16S rRNA sequence of *E. coli* was also analyzed by PCR (Zhu et al., 2007). The primer (forward: 5'-AAGAAGCTTGCTTCTTTGCTG-3'; reverse: 5'-GAGCCCGGGGATTTCACAT-3') was finally used to amplify the 16S rRNA gene. The cycling conditions were an initial step of 5 min at 94°C, 35 cycles of 30 s at 94°C, 40 s at 54°C, and 1 min at 72°C, followed by 10 min at 72°C.

Antibacterial Activity

The MIC of baicalin against the test bacteria was determined by broth dilution according to a modified method of Bandyopadhyay et al. (2008). With a sterile loop, 4 to 5 colonies of *E. coli* were picked up and suspended in 3 mL of LB broth and adjusted to 1×10^4 cfu/mL by dilution with LB broth. Fifty microliters of the bacteria suspension was added into each well of a sterile cell culture 96-well plate. Baicalin were diluted with LB broth to prepare a range of concentrations (1, 3, 6, 9, and 12 mg/mL); then, 100- μ L samples were added into each test well containing the bacteria. For the positive control, LB broth was added at 100 μ L in wells containing 50 μ L of bacteria. For the negative control, LB broth was added at 150 μ L in wells containing no bacteria. Finally, 15 μ L of resazurin (1 mg/mL) was added into each well. Three parallels of the well were made in each group. The MIC was defined as the lowest concentration of the samples at which the microorganism did not demonstrate visible growth after 24 h of incubation at 37°C. Microorganism growth was indicated by color change of the resazurin from blue to pink.

Bacteria Cell Wall Permeability

To examine the effect of baicalin on permeating of the *E. coli* cell wall, the methods of Zhou et al. (2016)

and Yap et al. (2015) were adapted. The MIC of baicalin was 4 mg/mL, and concentration of baicalin used in this trial was sub-MIC (1/2 MIC, 2 mg/mL). In the experimental group, 100 μ L of bacterial suspension (1×10^4 cfu/mL) was inoculated into 10 mL of LB broth containing baicalin (2 mg/mL). The negative and positive control groups had 100 μ L of adjusted suspension (1×10^4 cfu/mL) or baicalin (2 mg/mL), respectively. Three parallels were made in each group. Then the treated mediums were incubated at 37°C with agitation at 200 rpm. Bacterial cell wall permeability was determined at intervals of 0, 2, 4, 6, and 8 h using the AKP kit according to the instructions for *E. coli*.

Scanning Electron Microscopy Screening

In the bacteria cell wall permeability experiment, 5 mL of inoculum with a period of 8 h of incubation was subjected to scanning electron microscopy (Ganderton et al., 1992). A sterile coverslip (6 mm $\times$ 6 mm) was put into the inoculum and incubated at 37°C for 1 h. Then the coverslip was clamped with sterile forceps lightly and dried under natural conditions. Sample fixation was carried out with 2.5% glutaraldehyde for 1 h, and then stored at 4°C for 12 h. The coverslip was washed with PBS (pH 7.4) 2 to 3 times. Sequential exposure to different concentrations of alcohol (30% to 90%) caused further dehydration of the samples. The samples were subsequently subjected to critical point drying, sputter-coated with gold, and observed under an scanning electron microscope (S-4800, Hiachi High-Technologies Corporation, Takeda Hitachinaka-City, Japan). Three parallels were made in each group sample.

Antimicrobial Susceptibility Testing

The MIC of antimicrobial agents to the isolates in vitro were determined by an agar dilution method according to the standards described by the Clinical and Laboratory Standards Institution (M100-S25 and VET01-A4/VET01-S2). Concentration of the bacterial suspension was 1×10^4 cfu/mL, and concentration of baicalin used in this trial was sub-MIC (2 mg/mL). The following antimicrobials were assessed: ampicillin, penicillin, cefotaxime, cefoxitin, enrofloxacin, ciprofloxacin, ofloxacin, erythromycin, cillimycin, gentamicin, streptomycin, chloramphenicol, and tetracycline. *Escherichia coli* ATCC 25922 was used as the control strain.

Isolates were classified as either susceptible or resistant according to the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (M100-S25; ampicillin, penicillin, cefotaxime, cefoxitin, ciprofloxacin, ofloxacin, erythromycin, cillimycin, and streptomycin), and veterinary Clinical and Laboratory

Standards Institute (gentamicin, enrofloxacin, tetracycline, and chloramphenicol) (VET01-S2). *Escherichia coli* ATCC strain 25922 was used as the quality control in the antimicrobial susceptibility testing. The experiment was performed in triplicate.

Resistance DNA Extraction and Analysis

Extraction of DNA was carried out using the boiling method according to the Dei-Tutuwa's instructions for *E. coli* (Dei-Tutuwa et al., 2014). Frequently detected and studied genes, the plasmid-mediated quinolone resistance (PMQR) genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *aac(6')-Ib-cr*, *oqxA*, *oqxB*] and the extended-spectrum β -lactamases (ESBL; *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}), were chosen to amplify. All the oligonucleotide primers were synthesized by Zhuang et al. (2012) and Wang et al. (2013). The controls of strains that contained these genes came from the Pharmacological Laboratory of Northwest Agricultural and Forestry University. Polymerase chain reactions were performed on 0.5 mL of DNA using Taq DNA polymerase and 1 mM of each primer. All PCR products were analyzed by electrophoresis in 1% (wt/vol) agarose gel. Gels were then stained with ethidium bromide and photographed under UV light. All amplified samples were sequenced and compared with known sequences using the BLAST program at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/BLAST>).

RESULTS

Bacteriological Examination and Determination of MIC

Through the isolation, purification, and identification of pathogenic bacteria, 56 strains of *E. coli* were obtained from the 341 milk samples from 341 cows (Table 1). The antibacterial activity of baicalin to the isolated *E. coli* strains did not show variability because the MIC of baicalin to the *E. coli* strains was 4,000 μ g/mL for all.

Bacteria Cell Wall Permeability Assay

The activity of AKP representing the permeability of cell walls was illustrated in Figure 1. With the prolonged culture period, the enzyme activity was significantly increased from 1.246 to 2.377 U/100 mL. No significant changes were detected in either control group. This meant that AKP passed through the cell wall into the broth due to the effect of baicalin.

Table 1. *Escherichia coli* isolated from dairy herds located in 4 cities of Shaanxi province of China

City	No. of dairy herds ¹	No. of milk samples ²	<i>Escherichia coli</i> isolates ³
Baoji	BJ1/BJ2/BJ3/BJ4/BJ5/BJ6/BJ7	96 (9/12/12/15/21/16/11)	14 (1/2/2/2/3/3/1)
Weinan	WN1/WN2/WN3/WN4/WN5/WN6/WN7	90 (13/8/12/10/14/17/16)	13 (2/1/2/1/2/3/2)
Xi'an	XA1/XA2/XA3/XA4/XA5/XA6/XA7/XA8/XA9	114 (19/14/9/17/15/7/9/11/13)	22 (3/2/2/3/3/1/3/2/3)
Yangling	YL1/YL2/YL3	41 (15/12/14)	7 (3/2/2)

¹The letters indicate the city of sampling. BJ = Baoji; WN = Weinan; XA = Xi'an; YL = Yangling. The numbers after the letters represent the number of dairy herds.

²The first number indicates the total, and the numbers in parentheses indicate the sampling numbers corresponding to the farms in the second column.

³The first number indicates the total, and the numbers in parentheses show the *Escherichia coli* isolate numbers from the respective farms.

Scanning Electron Microscopy Screening

Electron microscopy observations further illustrated morphological changes in *E. coli* after incubation with MIC of baicalin for 8 h. Baicalin-untreated bacteria exhibited membrane structure integrity with a plump cell (Figure 2A). In contrast, the surface of *E. coli* showed depressions and damage following incubation with baicalin (Figure 2B), indicating that baicalin might attach and penetrate the cell membrane of bacteria, resulting in changes of membrane permeability.

Susceptibility Test of the *E. coli* Isolates

Resistance rates of the 56 *E. coli* isolates to the 13 antimicrobial agents before and after the baicalin

incubation are shown in Table 2. Before the incubation, susceptibility testing showed that the resistance rates of the β -lactams to the isolates were 7.1 to 100%. Penicillin and ampicillin, in particular, showed higher resistance rates. Only 4 isolates showed resistance to cefotaxime. The resistant strains of cefotaxime and cefoxitin were also resistant to penicillin and ampicillin. The resistance rates of the fluoroquinolones, enrofloxacin, ofloxacin, and ciprofloxacin, to the isolates ranged from 33.9 to 46.4%. Seventeen of the isolates had co-resistance to all the 3 fluoroquinolones. Fifteen of the isolates had multi-resistance to penicillin, ampicillin, enrofloxacin, ofloxacin, and ciprofloxacin. A high proportion of resistance to other antimicrobial agents commonly used on animal farms was also demonstrated in this study, including erythromycin (98.2%), cillimycin (94.6%), and achromycin (46.4%).

Under the interference of baicalin, the resistance rates of the β -lactams decreased from 1.7 to 14.3%. The most significant reductions were streptomycin (25.0%), ciprofloxacin (19.6%), and ampicillin (14.3%). Except for erythromycin and cillimycin, all of the antimicrobials had increased susceptibility to the isolates.

Effects of Baicalin on the Drug-Resistance Genes of *E. coli* Isolates

Overall, a total of 32 (57.14%, 32/56) *E. coli* isolates showed the presence of at least one ESBL gene and were included in the analysis (Table 3). *Bla*_{CTX-M} was the most prevalent ESBL gene (65.63%; 21/32), whereas *bla*_{TEM} and *bla*_{SHV} genes were present in 56.25% (18/32) and 21.88% (7/32) of ESBL-positive isolates, respectively. *Bla*_{OXA} was not detected. Eighteen (32.14%) isolates were detected as PMQR genes producing *E. coli*. The data set comprised 6 different PMQR genes: *oqx**B* (38.89%, 7/18), *oqx**A* (33.33%, 6/18), *aac*(6')-Ib-cr (22.22%, 4/18), *qnrD* (22.22%, 4/18), *qnrB* (16.67%, 3/18), *qnrS* (16.67%, 3/18%). *oqx**A* were most frequently observed together with *oqx**B*. *QnrA*, *qnrC*, and *qepA* were not detected.

Figure 1. Effects of baicalin on the cell wall permeability of *Escherichia coli*. The concentration of baicalin was 2 mg/mL, and the concentration of *Escherichia coli* was 1×10^2 cfu/mL. AKP = alkaline phosphatase. The error bars indicate the SD of each data set.

Figure 2. The morphological changes of *Escherichia coli* treated with baicalin. (A) The control group without the baicalin; (B) the experimental group treated with the baicalin. The concentration of baicalin was 2 mg/mL, and the concentration of *Escherichia coli* was 1×10^2 cfu/mL with a period of 8 h of incubation. The arrows indicate the depressions at the surface of *E. coli*.

Changes of drug resistance genes are presented in Figure 3. Many drug resistance genes were affected by baicalin. Among them, the loss of genes was as follows: *qnrD* 50% (2/4 isolates), *oqxA* 33.33% (2/6), *qnrS* 33.33% (1/3), *bla_{SHV}* 28.57% (2/7), *bla_{TEM}* 22.22% (4/18), *oqxB* 14.29% (1/7), and *bla_{CTX-M}* 9.5% (2/21). No changes in the *qnrB* and *aac(6')-Ib-cr* resistance genes were observed.

DISCUSSION

Antimicrobial therapy is commonly used for prevention and control of mastitis. Unfortunately, treatment failure is common because of antimicrobial resistance (Metzger and Hogan, 2013). In this study, we evaluated antibacterial activity and mechanisms of baicalin in vitro on *E. coli*. Meanwhile, the possible effects on the prevalent drug resistance genes were investigated for the available medication mechanisms (Ali et al., 2016).

After a routine identification test, 56 (16.42%) *E. coli* strains were obtained from the 341 mastitic milk samples, which had higher occurrence compared with previous reports from China (Ali et al., 2016). Bacteriostatic test in vitro showed that the MIC of baicalin to *E. coli* strains was 4 mg/mL, which indicated that baicalin had weak antibacterial activity against the *E. coli* strains. Similar research reported baicalin against antimicrobial susceptibility of *Helicobacter pylori* strains in vitro, of which the MIC₉₀ is 1.30 mg/mL (Wu et al., 2008). Furthermore, it was found that baicalin has antiviral efficacy (Huang et al., 2017).

Through incubation of baicalin at the sub-MIC for a short period, compared with the control group, the concentration of AKP increased from 1.246 to 2.377 U/100 mL in the experimental group. Alkaline phosphatase, a kind of enzyme located between the bacterial membrane and the rest of the bacterial wall, cannot be detected out of the bacteria cell wall normally.

Table 2. Numbers of drug-resistant *Escherichia coli* isolates before and after baicalin incubation¹

Antimicrobial		Number (%) of resistant isolates before baicalin incubation	Number (%) of resistant isolates after baicalin incubation
β-Lactam	Ampicillin	38 (67.9)	30 (53.6)
	Penicillin	56 (100)	49 (87.5)
	Cefoxitin	6 (10.7)	4 (7.1)
	Cefotaxime	4 (7.1)	3 (5.4)
Fluoroquinolone	Enrofloxacin	19 (33.9)	19 (33.9)
	Ciprofloxacin	26 (46.4)	15 (26.8)
	Ofloxacin	19 (33.9)	15 (26.8)
Other	Erythromycin	55 (98.2)	55 (98.2)
	Cillimycin	53 (94.6)	53 (94.6)
	Gentamicin	11 (19.6)	5 (8.9)
	Streptomycin	27 (48.2)	13 (23.2)
	Chloramphenicol	7 (12.5)	3 (5.3)
	Tetracycline	26 (46.4)	22 (39.3)

¹Isolates were classified as either susceptible or resistant according to the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, M100-S25; ampicillin, penicillin, cefotaxime, cefoxitin, ciprofloxacin, ofloxacin, erythromycin, cillimycin, and streptomycin), and veterinary CLSI (gentamicin, enrofloxacin, tetracycline, and chloramphenicol).

Figure 3. Changes of drug resistance genes before and after baicalin incubation. The concentration of baicalin was 2 mg/mL, and the concentration of *Escherichia coli* was 1×10^4 cfu/mL with a period of 8 h of incubation.

However, bacteria cell wall damage allows access of AKP out of the bacteria cell wall (Cox et al., 2001), and this hypothesis was supplemented by evidence of the bacteria cell wall permeability test in this study. It was suggested that the increase of permeability of the bacteria cell wall are indications of permeability of the bacteria cell wall (Hara and Yamakawa, 1995). The scanning electron microscopy study showed that the surface of *E. coli* was concave and shriveled following incubation with baicalin. These indicated that the structural integrity and stability of the bacterial cell wall as a permeability barrier were changed under the effect of baicalin. It is reported that baicalin showed inhibitory effects on various gram-negative bacteria in vitro, such as *Pseudomonas aeruginosa* (Luo et al., 2017) and *H. pylori* (Wu et al., 2008).

In the susceptibility test of the *E. coli* isolates, susceptibility of the isolates to the certain antimicrobial agents showed a tendency to improve compared with the pre-incubation isolates. Further detections that focused on the ESBL and PMQR drug resistance genes, found that baicalin affected the plasmid-carrying resistance genes. Interestingly, one or more resistance genes of some strains could not be detected following treatment with baicalin. Compared with isolates before baicalin treatment, drug resistance genes of 12 (30%, 12/40) strains changed. However, due to the limited samples and current stage of our research, there were no regular or certain findings. Baicalin can significantly decrease the MIC of amoxicillin and tetracycline against some *H. pylori* strains, possibly by mechanisms associated with decreasing *hefA* mRNA expression by report (Huang et al., 2015). In our present study, baicalin had the similar effects on the MIC of antimicrobial agents and ESBL and PMQR genes. Also, studies performed with resistance *E. coli* isolates from cows with clinical mastitis revealed a high prevalence of strains carrying

Table 3. Effect of baicalin on the drug-resistance genes of *Escherichia coli* isolates

Strain ¹	Before baicalin incubation	After baicalin incubation	Strain ¹	Before baicalin incubation	After baicalin incubation
BJ1	<i>bla</i> _{SHV} , <i>qnrB</i> , <i>qnrD</i>	<i>qnrB</i> , <i>qnrD</i>	XA3-1		<i>bla</i> _{TEM} , <i>qnrAB</i>
BJ2-1	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}	XA3-2		<i>bla</i> _{CTX-M}
BJ2-2	<i>bla</i> _{CTX-M} , <i>aac</i> (6')-Ib-cr	<i>bla</i> _{CTX-M} , <i>aac</i> (6')-Ib-cr	XA4-1		<i>bla</i> _{TEM} , <i>qnrS</i>
BJ3-1	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV}	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV}	XA4-2		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>qnrAB</i>
BJ3-2	<i>bla</i> _{CTX-M}	<i>bla</i> _{CTX-M}	XA4-3		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV}
BJ4-1	<i>qnrA</i>	<i>qnrA</i>	XA5-3		<i>bla</i> _{SHV} , <i>qnrB</i> , <i>qnrD</i>
BJ5-2	<i>bla</i> _{TEM}		XA6		<i>bla</i> _{TEM} , <i>qnrD</i>
BJ6-1	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>qnrD</i>	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>qnrD</i>	XA7-1		<i>aac</i> (6')-Ib-cr
BJ6-3	<i>qnrB</i> , <i>qnrD</i>	<i>qnrB</i> , <i>qnrD</i>	XA7-2		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}
WN1-2	<i>bla</i> _{TEM}		XA7-3		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>aac</i> (6')-Ib-cr
WN2	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}	XA8-1		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}
WN3-1	<i>qnrAB</i>	<i>qnrAB</i>	XA8-2		<i>bla</i> _{CTX-M}
WN4	<i>bla</i> _{TEM}		XA9-1		<i>bla</i> _{CTX-M}
WN5-2	<i>bla</i> _{CTX-M}	<i>bla</i> _{CTX-M}	XA9-2		<i>qnrS</i> , <i>aac</i> (6')-Ib-cr
WN6-1	<i>bla</i> _{CTX-M}	<i>bla</i> _{CTX-M}	XA9-3		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM} , <i>qnrAB</i>
WN6-3	<i>qnrB</i>	<i>qnrB</i>	YL1-2		<i>bla</i> _{SHV}
WN7-1	<i>bla</i> _{CTX-M}	<i>bla</i> _{CTX-M}	YL2-1		<i>qnrS</i>
XA1-2	<i>bla</i> _{TEM}		YL2-2		<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}
XA2-1	<i>bla</i> _{SHV}	<i>bla</i> _{SHV}	YL3-1		<i>bla</i> _{CTX-M}
XA2-2	<i>bla</i> _{CTX-M} , <i>bla</i> _{TEM}	<i>bla</i> _{SHV} , <i>bla</i> _{TEM}	YL3-2		<i>qnrAB</i>

¹The letters indicate the city of sampling. BJ = Baoji; WN = Wünan; XA = Xi'an; YL = Yangling. The 2 numbers after the letters in the column represent the number of dairy herds and the strain number, respectively. One number means only 1 strain was obtained in the farm.

either resistance genes or plasmid-mediated related resistance genes (Suojala et al., 2011). In the case of ESBL and PMQR, the drug resistance was stronger and more prevalent in the presence of resistance genes that could conjugal transfer. (Veldman et al., 2011; Liu et al., 2014). Therefore, our studies may be helpful to the clinical application of antimicrobial agents and treatment of diseases.

Multi-drug resistance inhibitors, which were studied for inhibiting drug transport protein activity of strains in recent years, could prevent drug efflux and recover intake of drugs in bacteria, thus eliminating multi-drug resistance (Wang et al., 2008). In accordance with an earlier study, *S. baicalensis* could eliminate the resistance plasmid (which produced β -lactamase) of *Staphylococcus aureus* (Chen, 2010). Furthermore, some studies reported that Shuanghuanglian could inhibit the activity of β -lactamases (He et al., 2012; Lei, 2014). In our study, effects of baicalin on the drug-resistance genes of *E. coli* isolates were observable. However, the elimination mechanism was unknown.

Traditional Chinese medicine is commonly used by people in China mainly as disease treatment and a supplement to mainstream health care. There is active interest in searching for new therapeutic approaches to deal with pathogenic bacteria. In particular, alternative modes of action against drug resistance bacteria have received special attention. In the present investigation, *S. baicalensis* inhibited *E. coli* isolates in vitro and reduced antimicrobial resistance of the isolated bacteria. Baicalin, a rich source to combat pathogenic bacteria and reduce development of antimicrobial resistance in the dairy industry, was shown to be present in traditional Chinese medicinal herbs.

ACKNOWLEDGMENTS

This work was supported by the Key Science and Technology Program of Shaanxi Province, China (grant no. 2014K02-05-01).

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