Short communication: Detection and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from subclinical bovine mastitis cases in China

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ABSTRACT

This study investigated the antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from cases of subclinical bovine mastitis in China, as well as resistance mechanisms and virulence genes encoding adhesins and toxins. We determined antimicrobial susceptibility using the disk diffusion method, and analyzed resistance, adhesin, and toxin genes using PCR. We confirmed MRSA in 73 of 498 (14.7%) *Staph. aureus* isolates recovered from subclinical mastitic milk samples. All isolates were positive for *mecA*. The MRSA isolates showed high resistance to penicillin (100.0%), gentamicin (100.0%), and tetracycline (98.6%). All MRSA isolates harbored resistance genes *blaZ* (penicillin), *aacA/aphD* (gentamicin), and *tetM* (alone or in combination with *tetK*, tetracycline). Moreover, all isolates carried the adhesin genes *fmbpA*, *clfA*, *clfB*, *cna*, *sdrE*, and *map/eap*, and most carried *sdrC* (98.6%), *sdrD* (95.9%), *bbp* (94.5%), and *ebpS* (80.8%). The toxin genes *seh*, *hla*, and *hld* were present in all isolates, and most isolates carried *sea* (71.2%), *seg* (84.9%), *sei* (82.2%), *lukE-lukD* (97.3%), and *hlg* (72.6%). These findings of high-level resistance to antimicrobials commonly used in dairy cattle should lead to calls for antibiogram analysis before antimicrobial therapy. The high frequency of adhesin and toxin genes in MRSA indicates their potential virulence in bovine mastitis in China.

Key words: methicillin-resistant *Staphylococcus aureus*, bovine mastitis, antimicrobial resistance, virulence gene

Short Communication

Bovine mastitis is a highly prevalent and costly disease for the dairy industry worldwide (Yang et al., 2018). *Staphylococcus aureus* is a major etiological pathogen that causes bovine mastitis. Antimicrobial therapy is a primary tool for controlling staphylococcal mastitis (Wang et al., 2018), but the cure rate is low because of increasing antimicrobial resistance developed by this pathogen, especially methicillin-resistant *Staph. aureus* (MRSA). Indeed, MRSA has shown resistance to all types of β -lactam antibiotics currently available for mastitis therapy (Vanderhaeghen et al., 2010).

Methicillin-resistant *Staph. aureus* are considered a serious threat to public health because they can be easily spread through healthy carriers, and they express a large number of virulence factors, including toxins, cell wall-associated adhesins, and secreted exoproteins, making infection more likely (Herrera et al., 2016). Recently, the number of publications describing the detection of MRSA in milk or dairy products has grown rapidly worldwide (Holmes and Zadoks, 2011; Seixas et al., 2014; Al-Ashmary et al., 2016; Guimarães et al., 2017; Klibi et al., 2018). However, little is known about the virulence gene profiles of MRSA from bovine mastitis; in China, only a few reports exist of some virulence determinants of limited MRSA isolates (Pu et al., 2014; Li et al., 2017b; Wang et al., 2018). We designed the current study to investigate the antimicrobial susceptibility of, resistance mechanisms implicated in, and virulence genes encoding adhesins and toxins of MRSA isolates from cases of subclinical bovine mastitis in China.

The MRSA strains investigated in this study were confirmed from 498 *Staph. aureus* isolates recovered from 3,136 cases of subclinical bovine mastitis. The mastitic milk samples were obtained from 102 commercial dairy herds in 19 provinces in China from August

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2013 to April 2018, as described in our previous study (Yang et al., 2018). We identified *Staph. aureus* by morphological characterization and biochemical testing, as previously described (Cressier and Bissonnette, 2011). The MRSA strains were confirmed by the cefoxitin (30 µg) disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI; CLSI, 2013).

We performed antimicrobial susceptibility testing using the disk diffusion method on Mueller–Hinton agar (Oxoid, Basingstoke, UK) according to CLSI criteria (CLSI, 2013). We applied the following panel of antimicrobial agents (Oxoid): penicillin (10 units), gentamicin (10 µg), tetracycline (30 µg), nitrofurantoin (300 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), quinupristin/dalfopristin (15 µg), and linezolid (30 µg). We used *Staph. aureus* ATCC 25923 as a positive control strain.

We extracted bacterial genomic DNA using the Bacterial DNA Kit (Omega Bio-Tek, Norcross, GA; Yang et al., 2018). The corresponding resistance genes for methicillin (*mecA* and *mecC*), penicillin (*blaZ*), gentamicin (*aacA/aphD*), and tetracycline (*tetK* and *tetM*) were searched using PCR as previously described (Paterson et al., 2012; Yang et al., 2016). We also performed PCR assays as described previously for adhesin genes encoding fibronectin bind protein (FnBPA; *fnbpA*), clumping factor (ClfA and ClfB; *clfA* and *clfB*), collagen binding protein (Cna; *cna*), serine-aspartic acid repeat proteins (SdrC, SdrD, and SdrE; *sdrC*, *sdrD*, and *sdrE*), bone sialoprotein-binding protein (Bbp; *bbp*), elastin-binding protein (EbpS; *ebpS*), and major histocompatibility complex class II analogous protein/extracellular adherence protein (Map/Eap; *map/eap*; Peacock et al., 2002). In addition, we evaluated the toxin genes encoding staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sen*, *seo*, and *sem*), toxic shock syndrome toxin-1 (TSST-1; *tst*), exfoliative toxins (*eta* and *etb*), leukocidins (PVL, LukE-LukD, and LukM; *lukPV*, *lukE-lukD*, and *lukM*), hemolysins (α-hemolysin, β-hemolysin, γ-hemolysin, δ-hemolysin; *hla*, *hlb*, *hld*, and *hlg*), and epidermal cell differentiation inhibitor (EDIN; *edin*) using PCR and following a method described previously (Jarraud et al., 2002). The PCR products were analyzed using 1.2% agarose gel electrophoresis.

Among 498 *Staph. aureus* isolates, we identified 73 (14.7%) as MRSA based on the cefoxitin disk diffusion method. The antimicrobial resistance profiles of the MRSA isolates are listed in Table 1. All MRSA isolates were resistant to penicillin and gentamicin, but susceptible to nitrofurantoin, trimethoprim/sulfamethoxazole, quinupristin/dalfopristin, and linezolid.

Resistance to tetracycline was detected at high rate in 72 (98.6%) isolates. Only 2 isolates were resistant to chloramphenicol (2.7%).

All MRSA isolates carried >4 of the tested resistance genes (Table 1). The resistance genes *mecA*, *blaZ*, and *aacA/aphD* were present in all isolates, but *mecC* was not found in any isolates. All isolates harbored the tetracycline resistance gene *tetM* (100.0%), either alone or in combination with *tetK* (95.9%), including 1 (1.4%) tetracycline-susceptible isolate.

The presence and distribution of adhesin and toxin genes in the MRSA isolates are listed in Table 1. All MRSA isolates harbored the adhesin genes *fnbpA*, *clfA*, *clfB*, *cna*, *sdrE*, and *map/eap*, and most contained *sdrC* (98.6%, 72/73), *sdrD* (95.9%, 70/73), *bbp* (94.5%, 69/73), and *ebpS* (80.8%, 59/73). All tested isolates contained the toxin genes *seh*, *hla*, and *hld*, and most contained *sea* (71.2%, 52/73), *seg* (84.9%, 62/73), *sei* (82.2%, 60/73), *lukE-lukD* (97.3%, 71/73), and *hlg* (72.6%, 53/73). Fewer isolates contained *seb* (19.2%, 14/73), *sec* (19.2%, 14/73), *sen* (30.1%, 22/73), *seo* (30.1%, 22/73), *sem* (28.8%, 21/73), *tst* (28.8%, 21/73), *lukPV* (2.7%, 2/73), and *hbl* (23.3%, 17/73). None of the 73 MRSA isolates carried the toxin genes *sed*, *see*, *sej*, *eta*, *etb*, or *edin*.

Although the prevalence of MRSA in bovine mastitis is generally low (Bardiau et al., 2013; Seixas et al., 2014), the emergence and spread of MRSA is becoming a major animal and public health problem worldwide (Parisi et al., 2016; Klibi et al., 2018). Previous studies have shown that the occurrence of MRSA from bovine mastitis varied from 1.4 to 47.6% in China (Pu et al., 2014; Wang et al., 2018). In the present study, 14.7% of *Staph. aureus* isolates were identified as MRSA. Our findings were in line with studies conducted in other countries, which revealed that the prevalence of MRSA was 9.3% in Belgium, 17.2% in Turkey, 13.9% in Korea, and 6.6% in Tunisia (Türkyilmaz et al., 2010; Vanderhaeghen et al., 2010; Song et al., 2016; Klibi et al., 2018). In contrast, MRSA has been detected in bovine mastitis more frequently in India (48.7%; Mistry et al., 2016). These variations in frequencies may be due to different sample sizes, different seasons, and geographical discrepancies (Klibi et al., 2018).

In the current study, all MRSA isolates harbored the methicillin-resistant gene *mecA*, but no isolate carried *mecC*. Some *mecC*-positive MRSA isolates from bovine milk have been reported in different countries (Prenafeta et al., 2014; Herrera et al., 2016). However, to the best of our knowledge, *mecC*-positive MRSA from bovine mastitis in China has not been detected to date. In the current study, all MRSA isolates showed resistance to penicillin and gentamicin, and most displayed

Table 1. Antimicrobial resistance and virulence genes of 73 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from subclinical bovine mastitis cases¹

Resistance pattern	Resistance genes	Virulence genes		Isolates, no.
		Adhesins	Toxins	
PEN, GEN, TET, CHL	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnE</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukPV</i> , <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>hlg</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	18
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i>	3
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seh</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	6
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seh</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	4
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	3
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>hlg</i>	3
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>hlg</i>	5
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fibA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdnC</i> , <i>sdnD</i> , <i>sdnE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ebp</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>seo</i> , <i>sem</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hly</i> , <i>hld</i>	1

Continued

Table 1 (Continued). Antimicrobial resistance and virulence genes of 73 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from subclinical bovine mastitis cases¹

Resistance pattern	Resistance genes	Virulence genes		Isolates, no.
		Adhesins	Toxins	
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrD</i> , <i>sdrE</i> , <i>bbp</i> , <i>map/ eap</i>	<i>sea</i> , <i>seh</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hnb</i> , <i>hld</i> , <i>hlg</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrD</i> , <i>sdrE</i> , <i>bbp</i> , <i>map/ eap</i>	<i>sea</i> , <i>seh</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrD</i> , <i>sdrE</i> , <i>bbp</i> , <i>map/ eap</i>	<i>seh</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>sco</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i>	2
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrD</i> , <i>sdrE</i> , <i>bbp</i> , <i>map/ eap</i>	<i>seh</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>sco</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrD</i> , <i>sdrE</i> , <i>bbp</i> , <i>ebpS</i> , <i>map/ eap</i>	<i>seh</i> , <i>sec</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>sen</i> , <i>sco</i> , <i>sem</i> , <i>tst</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN, TET	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrE</i> , <i>ebpS</i> , <i>map/ eap</i>	<i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hnb</i> , <i>hld</i> , <i>hlg</i>	1
PEN, GEN	<i>mecA</i> , <i>blaZ</i> , <i>aacA/aphD</i> , <i>tetK</i> , <i>tetM</i>	<i>fmbA</i> , <i>clfA</i> , <i>clfB</i> , <i>cna</i> , <i>sdrC</i> , <i>sdrD</i> , <i>sdrE</i> , <i>ebpS</i> , <i>map/ eap</i>	<i>sea</i> , <i>seg</i> , <i>seh</i> , <i>sei</i> , <i>lukE-lukD</i> , <i>hla</i> , <i>hld</i> , <i>hlg</i>	1

¹PEN = penicillin; GEN = gentamicin; TET = tetracycline; CHL = chloramphenicol.

resistance to tetracycline. All of these isolates harbored the resistance-related genes *blaZ* (penicillin), *aacA/aphD* (gentamicin), and *tetM* (tetracycline, alone or in combination with *tetK*). Similar high-level phenotypic and genotypic resistance to these antimicrobial agents has been frequently observed in MRSA from bovine mastitis in other studies (Türkyilmaz et al., 2010; Bardiau et al., 2013; Silva et al., 2014). This high resistance rate and the presence of resistance-related genes could be explained by the frequent use of these antimicrobials in dairy cattle in China (Li et al., 2017a; Liu et al., 2017). Interestingly, 1 tetracycline-susceptible MRSA strain carried both *tetK* and *tetM* genes. This discrepancy may have been due to the lack of expression of the resistance genes (Hammad et al., 2014).

Staphylococcus aureus can express various adhesins that are essential for bacterial colonization to host cells (McCarthy and Lindsay, 2010). Fibronectin bind protein (*fmbpA*), clumping factors (*clfA* and *clfB*), and collagen binding protein (*cna*) are important adhesins for binding to host cells, colonization, and invasion (Aslantaş and Demir, 2016). In the current study, all MRSA isolates harbored the adhesin genes *fmbpA*, *clfA*, *clfB*, and *cna*, similar to previous studies in China and other countries (Aslantaş and Demir, 2016; Locatelli et al., 2017; Wang et al., 2018). Putative cell-surface proteins encoded by *sdrC*, *sdrD*, and *sdrE* could recognize adhesive matrix molecules and promote *Staph. aureus* adhesion to host cells (Tung et al., 2000; Corrigan et al., 2009; Yu et al., 2012). In the current study, we found that all MRSA carried *sdrE*, and most of them contained *sdrC* and *sdrD*, in agreement with previous studies (Yu et al., 2012; Cheraghi et al., 2017). In addition, we detected the *bbp* and *ebpS* genes in 94.5 and 80.8% of the MRSA isolates, respectively; Bbp and EbpS can mediate the binding of *Staph. aureus* to surface proteins or soluble elastin peptides in host cells (Aung et al., 2011). Our findings agreed with those reported by others with respect to the presence of *bbp* and *ebpS* (Aung et al., 2011; Tang et al., 2013). Moreover, all MRSA isolates in the current study carried *map/eap*, in agreement with previous human studies (Peacock et al., 2002; Cheraghi et al., 2017). Adhesive proteins Map and Eap have been associated with the adhesive interactions of *Staph. aureus* with a broad spectrum of host molecules (Hussain et al., 2002). To the best of our knowledge, this is the first report of *map/eap* in MRSA recovered from bovine mastitis in China.

In addition to adhesins, *Staph. aureus* can produce a wide range of toxins involved in the invasion of host cells and the evasion of immune response (Seixas et al., 2014). We determined the presence of toxin genes among the 73 MRSA isolates in the current study. It has been suggested that toxins SEs and TSST-1 may

play a role in the development of bovine mastitis by providing an attractive environment for staphylococcal colonization (Artursson et al., 2016). In the current study, all MRSA isolates harbored at least 2 staphylococcal enterotoxin genes; *seh*, *seg*, *sei*, and *sea* were the most frequently detected. These findings were in accordance with those of other reports (Yu et al., 2012; Song et al., 2016; Klibi et al., 2018; Wang et al., 2018). Moreover, 28.8% of the isolates contained *tst* in this study, in line with previous studies in China (Wang et al., 2016; Zhang et al., 2018). Leukotoxins and hemolysins are thought to play important roles in the initiation or exacerbation of mastitis. They are 2-component toxins that kill erythrocytes, phagocytes, or both by forming pores in host cell membranes (Fueyo et al., 2005; Chalmeau et al., 2011). Similar to previous studies (Fueyo et al., 2005; Al-Ashmawy et al., 2016; Wang et al., 2018), we found that most of the MRSA contained *lukE-lukD* (97.3%). We observed *lukPV* in only 2.7% of isolates, although it has been reported that *lukPV* commonly presents at a high prevalence among methicillin-sensitive isolates (Wang et al., 2018). Furthermore, all MRSA isolates carried *hla* and *hld*, and many carried *hlg* (72.6%) and *hnb* (23.3%). These findings were in agreement with other recent studies, which reported that *hla* and *hld* genes were frequently observed in *Staph. aureus* (Xu et al., 2015; Wang et al., 2016).

In conclusion, although the prevalence of MRSA was low, the isolates in the current study exhibited a high rate of phenotypic and genotypic resistance to antimicrobials commonly used in dairy cattle in China. The potential risk of MRSA transmission via the food chain cannot be ignored. In addition, the high occurrence of all adhesin genes tested in this study and the toxin genes *sea*, *seg*, *seh*, *sei*, *lukE-lukD*, *hla*, *hld*, and *hlg* in MRSA indicate their pathogenic potential in bovine mastitis in China. However, bovine mastitis caused by MRSA cannot be explained by the action of an individual virulence factor (Peacock et al., 2002). Further studies need to be conducted to explore the combined action of these factors in MRSA pathogenesis.

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