



Bovine mastitis *Staphylococcus aureus*: Antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China



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ABSTRACT

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dairy animals is of great concern for livestock and public health. The aim of present study was to detect new trends of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) towards antibiotic susceptibility, resistance genes and molecular typing by methods of disc diffusion, multiplex PCR assay and multilocus sequence typing (MLST). A total of 219 *S. aureus* strains were isolated from bovine mastitis cases from six provinces of China, including 34 MRSA strains. The results revealed that more than 70% isolated strains showed resistance to various antibiotics, and multiple-drugs resistance to more than five categories of antibiotics was found more common. The *ermC* was the most prevalent resistance gene, followed by other genes; however, *ermA* was the least frequently detected gene. Twenty-eight *mecA*-negative MRSA and six *mecA*-positive MRSA strains were detected, and in which three strains were ST97-MRSA-IV, others were ST965-MRSA-IV, ST6-MRSA-IV and ST9-MRSA-SCCmec-NT. The *mecA*-negative MRSA strains were found resistant to most of the antibiotics, and harbored *aac(6')/aph(2'')*, *aph(3')-III* and *tetM* genes higher than MSSA strains. The resistance to most of the antibiotics was significantly higher in MRSA than in MSSA strains. The MLST profiles showed that these strains mainly belonged to CC5, CC398, CC121 and CC50 lineage, especially within ST97 and ST398, while some novel sequence types (ST2154, ST2165 and ST2166) were identified and deposited in the MLST database. This indicates that the resistance of *S. aureus* is becoming more complicated by changes in multi-drug resistance mechanism and appearance of *mecA*-negative MRSA isolates, and importantly, MRSA-IV strains in different MLST types are emerging.

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1. Introduction

Staphylococcus aureus is the most common etiological pathogen of contagious bovine mastitis and it has the potential to develop resistance to almost all the antimicrobial agents (Barkema et al., 2009; Hiramatsu et al., 2001). These organisms are well known for their tolerance to wide range of adverse circumstances. This tolerance is narrated to the diverse genetic capabilities of these microbes, emergence of methicillin-resistant *Staphylococcus aureus*

(MRSA) strains, and to the formation of biofilms in the host, which influences *S. aureus* pathogenesis in mastitis, evades the host immune response and crafts multi-drugs resistance (Brady et al., 2011; Kenar et al., 2012). It has also been reported that the relative higher incidence of *S. aureus* mastitis in China than other countries, its treating difficulty and chronic mastitis in dairy herds may be attributed to these properties of the organism (He et al., 2014; Li et al., 2011). Antibiotic resistance of *S. aureus* is widely spreading accompanied with extensive utilization of antibacterial agents in bovine mastitis. This antimicrobial resistance developed by the pathogens is one of main reasons of low cure rate of mastitis (Barkema et al., 2006; Gao et al., 2012). Besides that, more important is the emergence of multiple drugs resistant (MDR) by *S. aureus* strains, which has become a major challenge in the

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treatment of bovine mastitis and has been developed into public health issue (Davies, 1994). MRSA strains have become the pathogen of great concern in human beings, and recently in dairy animals. The emergence of MRSA strains has considerably increased the potential threats of zoonosis (Juhász-Kaszanyitzky et al., 2007; Loncaric et al., 2014), as MRSA has been reported in human as well as veterinary medicine (Leonard and Markey, 2008; Otter and French, 2010). MRSA from livestock may be the causal agents for human MRSA infections (Lee, 2003), and it has been reported that animals might be the potential reservoirs of MRSA strains for human beings (Garcia-Alvarez et al., 2011; Verkade and Kluytmans, 2014). To approach the appropriate treatment and control measures for bovine mastitis, it is important to study the antimicrobial resistance mechanism and epidemiology of *S. aureus* infections.

To date, published data on antibiotic resistance genes and molecular typing of MRSA and MSSA strains isolated from bovine mastitis in China, especially by using multilocus sequence typing (MLST), is scarce. Therefore, the present study was designed with the objective to analyze *S. aureus* strains from bovine mastitis isolated from diverse geographical regions of China.

2. Materials and methods

2.1. Isolation and identification of *S. aureus* strains

A total of 219 *S. aureus* strains were isolated from bovine mastitis cases during March, 2010 to August, 2013. This included 143 strains isolated from sixteen herds scattered in different areas of the Xinjiang Uygur autonomous region marked XJ or XJH, 13

strains isolated from Beijing, Shanxi province (SX, 18 strains), Shandong province (SD, 8 strains) and Inner Mongolia autonomous region (IM, 17 strains), and 20 strains were donated by Professor Hu Songhua from Zhejiang University, collected from Zhejiang province (JH, HZ and NB) (Fig. 1). *S. aureus* strains were cultured on Columbia sheep blood agar plates and were presumptively identified as *S. aureus* according to the following scheme: gram-positive cocci, hemolytic on sheep blood agar, catalase-positive and coagulase-positive. The presumptive identification was confirmed by API ID32 Staph (bioMérieux, France) and PCR amplification of the *nuc* gene of *S. aureus* (Brakstad et al., 1992). The isolates were stored at -80°C in Luria–Bertani (LB) broth (Invitrogen, Beijing, China) with 50% (v/v) glycerol prior to use.

2.2. Antibiotic susceptibility of isolates

Disc diffusion test was used to test the susceptibility of *S. aureus* isolates according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2009). Ten commonly used antibiotics including erythromycin (15 μg), clindamycin (2 μg), ceftioxin (30 μg), penicillin (10 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), doxycycline (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and gentamicin (10 μg) (BD BBL, Sparks, MD, USA) were tested. *S. aureus* ATCC 25923 and ATCC 29213 were used as the quality control. Moreover, the erythromycin-resistant strain was tested for induction of clindamycin resistance as described previously (Steward et al., 2005). The ceftioxin-resistant strains were identified using microdilution test



Fig. 1. Map of China showing location of the six provinces from where bovine *S. aureus* strains were collected.

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