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Phenotypic and genotypic characterization of antimicrobial resistance profiles in *Streptococcus dysgalactiae* isolated from bovine clinical mastitis in 5 provinces of China

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ABSTRACT

Bovine mastitis is among the most prevalent and costly diseases of dairy animals and is caused by a variety of bacterial pathogens including *Streptococcus dysgalactiae*. However, comprehensive studies reporting the prevalence and antimicrobial resistance profiles of *S. dysgalactiae* isolated from bovine mastitis are scarce. Therefore, this study was to investigate the occurrence of *S. dysgalactiae* associated with bovine clinical mastitis, to assess their antimicrobial resistance profiles, and to analyze the phenotypic and genotypic profiling of resistant isolates. In total, 1,180 milk samples were collected from dairy cows with clinical mastitis belonging to 74 commercial dairy herds located in 14 provinces of China from January 2014 to May 2016. Overall *S. dysgalactiae* isolates were recovered from 88 (7.5%) of the mastitic milk samples. The antimicrobial susceptibility of these isolates was tested against 8 antimicrobial agents by using minimum inhibitory concentrations. Results showed that 82 (93.2%) isolates expressed resistance to more than one antimicrobial agent. Antimicrobial resistance was highest against kanamycin (89.8%), sulfonamide (83.0%), and streptomycin (58.0%), which can be attributed to the intrinsic resistance for most of *Streptococcus* spp. against those antimicrobial substances. Strikingly, 30 (34.1%) and 12 (13.6%) isolates were found resistant to cephalixin and ceftriaxone, respectively. *Bla*TEM, *ermB*, and *tetM* were the most prevalent resistance genes. All isolates carried at least one of all tested resistance genes. Also, 1.1, 12.5, 18.2, 36.4, and 31.8% of isolates were positive for at least one tested resistance gene in 1, 2, 3, 4, or 5 classes of antimicrobials. Survival analysis showed a

significant association between *ermB* and survival of the *S. dysgalactiae* isolates at increasing erythromycin concentrations. No other statistically significant associations were observed between the phenotypic and genotypic resistance profiles. This study concludes a considerable prevalence of *S. dysgalactiae* associated with bovine mastitis in dairy herds of China and these isolates exhibited high resistance rates to tested antimicrobials, coupled with high occurrence of resistance genes. Both the prevalence of *S. dysgalactiae* and their antimicrobial resistance profiles strongly varied among dairy herds, demonstrating the need for antimicrobial susceptibility surveillance at the herd level to ensure optimal therapeutic results.

Key words: *Streptococcus dysgalactiae*, antimicrobial resistance, bovine clinical mastitis

INTRODUCTION

Bovine mastitis, inflammation of the mammary gland, is still the most prevalent disease in dairy cattle. The inflammatory reaction primarily occurs in response to bacterial IMI and impairs milk quality (Petrovski et al., 2006; Halasa et al., 2007). This disease also accounts for the highest proportion of antimicrobial usage on a dairy farm (Saini et al., 2012; Kuipers et al., 2016; Stevens et al., 2016). It is estimated that 60 to 70% of all antimicrobials used on dairy farms is applied for the prevention and control of mastitis, of which roughly half is related to the treatment of clinical mastitis (Kuipers et al., 2016; Stevens et al., 2016). Mastitis is characterized by clinical signs of inflammation and alterations in the appearance of milk (i.e., clinical mastitis) or it may be without any visual abnormalities (i.e., subclinical mastitis). Clinical and subclinical mastitis also affect animal welfare (Peters et al., 2015).

Apart from one recent research work in which the prevalence of the most common mastitis pathogens was

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studied in bulk milk samples collected from 894 Chinese dairy farms (Bi et al., 2016) and one recent report about the incidence of clinical mastitis and distribution of pathogens on 161 large Chinese dairy farms (Gao et al., 2017), no other information is available on the prevalence and distribution of mastitis pathogens in Chinese dairy farms. Of particular interest in the first study was the high prevalence of *Streptococcus dysgalactiae*-positive bulk milk samples; *S. dysgalactiae* was detected in 72.3% of the bulk milk samples (Bi et al., 2016). It was noteworthy that the prevalence of *S. dysgalactiae* was significantly higher than the prevalence of other major mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* (Gao et al., 2017), as well as in most other countries including Norway, Belgium, France, and Thailand (Piepers et al., 2007; Whist et al., 2007; Botrel et al., 2010; Leelahapongsathon et al., 2014). Still, bacteria in bulk milk do not solely originate from infected quarters. Environmental contamination or insufficient hygiene and poor preservation conditions can also cause positive bulk milk samples (Elmoslemany et al., 2009a,b). To get better insights in the exact relevance of *S. dysgalactiae* for the udder health in dairy cows in China, studies on the prevalence of *S. dysgalactiae* in clinical mastitis are required.

Despite the increasing pressure to reduce antimicrobial use in food-producing animals, antimicrobials still have important benefits for animal health and are still an essential tool in mastitis control programs (Middleton et al., 2014). Failure or success of treatment of clinical mastitis depends on several factors including those related to the cow such as age or parity, stage of lactation (DIM), SCC, and clinical mastitis history, and those related to the pathogen such as virulence and antimicrobial susceptibility (Sol et al., 2000; Taponen et al., 2003; Barkema et al., 2006). Antimicrobial resistance surveillance studies are imperative to choose the most appropriate antimicrobial therapies and to reduce the risk for further development and spread of antimicrobial resistance through lateral transfer of resistance genes or direct transfer of resistant pathogens. Some information is available on the prevalence of phenotypic antimicrobial resistance in *S. dysgalactiae* (Guérin-Faubleé et al., 2002; Bengtsson et al., 2009; McDougall et al., 2014; Petrovski et al., 2015), though no studies have comprehensively investigated the genotypic antimicrobial resistance in *S. dysgalactiae*.

The main objective of the current study was to estimate the prevalence of clinical mastitis caused by *S. dysgalactiae* in Chinese dairy herds and to determine the prevalence of both phenotypic and genotypic antimicrobial resistance profiles of the collected *S. dysgalactiae* isolates. Additionally, results obtained by phenotypic

and genotypic profiling of antimicrobial resistance were compared.

MATERIALS AND METHODS

Data and Sample Collection

Streptococcus dysgalactiae isolates included in this study were collected from milk samples of dairy cows with clinical mastitis ($n = 1,180$). In total, 74 dairy farms located in 14 different provinces were involved in the study (Beijing = 23 milk samples; Tianjin = 85 samples; Hebei = 196 samples; Shandong = 87 samples; Shaanxi = 26 samples; Shanxi = 48 samples; Inner Mongolia = 321 samples; Guangdong = 98 samples; Shanghai = 47 samples; Henan = 17 samples; Anhui = 78 samples; Heilongjiang = 56 samples; Jilin = 25 samples; Liaoning = 73 samples). On each farm, all the dairy farms veterinarians were requested to report the signs of clinical mastitis cases along with aseptically collected milk samples between January 2014 and May 2016. In case that only one quarter was affected, sample was taken from the quarter showing signs of clinical mastitis. If 2 or more quarters showed clinical signs at the same time, a composite milk sample from all quarters showing clinical signs [any abnormal aspect of the milk (flakes, clots, and a watery or other unusual appearance) with or without other visible abnormalities of the udder (e.g., redness, swelling, firmness)] was collected. Sample materials were provided and sampling procedures were explained in light of National Mastitis Council (NMC, 1999) instructions as well as the importance of an aseptic sampling procedure. Samples taken from the same cow within 3 mo from a previous case were excluded from the analysis. All the milk samples for bacteriological culturing were transported in ice boxes to our Bovine Mastitis Research Laboratory at the College of Veterinary Medicine, China Agricultural University, Beijing, China.

Microbiology

A volume of 30 μ L from each of the composite milk samples was plated on a tryptic soy agar (TSA; Oxoid, Basingstoke, UK) containing 5% defibrinated sheep blood (Land Bridge Technology, Beijing, China) and incubated aerobically at 37°C for 24 h. Subsequently, the identification of *Streptococcus dysgalactiae* was carried out by Gram staining, colony morphology, and biochemical tests. Streptococci (catalase-negative) were differentiated from staphylococci (catalase-positive) using catalase test. Isolates of the *Streptococcus-Enterococcus* group were differentiated as esculin-positive (*Streptococcus uberis* and other esculin-positive cocci)

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