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RESEARCH ARTICLE

Molecular Epidemiology and Characteristics of *Streptococcus agalactiae* Isolated from Bovine Mastitis in Large Dairy Herds of China

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ABSTRACT

Streptococcus agalactiae is one of the important causative agents of bovine mastitis. Studies on molecular epidemiology, virulence factors and antimicrobial susceptibility profiles of *S. agalactiae* isolates from mastitis in China are scarce. Thus, this study was carried out to investigate prevalence of *S. agalactiae* associated with subclinical mastitis, to determine antimicrobial susceptibility profile, and to analyze their phenotypic and genotypic profiles. Capsular serotypes and genotypes by multilocus serotyping and virulence genes (*hemolysin III*, *C-β protein*, *C-α protein*, *surface protein rib*, *hyaluronate lyase*, and *C5a peptidase*) were determined using molecular assays. Additionally, susceptibility of *S. agalactiae* isolates to antimicrobial agents was accessed through standard disc diffusion method. A total of 2225 milk samples were collected from 21 large dairy herds located in 10 provinces of China. Overall, 133 (14.1%) *S. agalactiae* isolates were recovered from 946 (42.5%) subclinical mastitis milk samples. Serotype *Ia* and ST103 were the most prevalent serotypes and genotypes. Five of the six virulence genes were detected in 22.6% isolates with only two virulence genes and 6.8% of isolates with three genes. There were no significant geographical differences in the distribution of virulence genes. Moreover, 63.9% of *S. agalactiae* exhibited resistance to levofloxacin, 3.8% isolates were resistant to cefepime, 2.3% were resistant to chloramphenicol and ampicillin. Additionally, two ampicillin/cefotaxime/ levofloxacin-resistant isolates, one vancomycin-resistant isolate and one ampicillin/cefotaxime-resistant isolate were identified. This study concludes high prevalence of *S. agalactiae* from subclinical mastitis carrying virulence genes and mainly belonging to Serotype *Ia* and ST103.

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INTRODUCTION

Bovine mastitis is an inflammatory response in the mammary gland of dairy cows typically caused by physical, chemical and microbiological factors (Rahularaj *et al*., 2019). More than 150 bacteria have been discovered from mastitic milk, and some of them are capable of developing severe bovine mastitis (Ali *et al*., 2008; Nonnemann *et al*., 2019). *Streptococcus agalactiae* (*S. agalactiae*) is the most common mastitis pathogens imparting serious economic losses considerable reduction in milk production (Maisano *et al*., 2018; Svennesen *et al*., 2019). Conditions such as improper management and poor sanitation are predisposing factors of *S. agalactiae* in dairy herds.

S. agalactiae is also known as group B *Streptococcus* (Raabe and Shane, 2019), and is recognized as a pathogen that can infect a variety of hosts including human, cows, fish, rabbits and other animals (Ren *et al*., 2014; Eisenberg *et al*., 2017; Li *et al*., 2018). Based on the diversity of capsular polysaccharides genes 10 serotypes (*Ia*, *Ib* and *II-IX*) of *S. agalactiae* have been reported (Imperi *et al*., 2010). These serotypes may differ in geographic distribution or preferred host. The pathogenicity of *S. agalactiae* is specified as serotypes, with serotype *Ia* more pathogenic than *III* (Chu *et al*.,

2016). Some isolates might possess zoonotic capacities, allowing infection of humans and a variety of animal hosts (Lyhs *et al*., 2016).

In China, more than 80 million cattle and buffaloes are farmed to provide high-quality milk and beef products. The most frequently suffered mastitis and isolated pathogens in China are *Escherichia coli*, *Klebsiella* spp., non-aureus staphylococci, *Streptococcus* spp. and *Staphylococcus aureus* (Ali *et al*., 2016; Ali *et al*., 2017; Gao *et al*., 2017). *S. agalactiae* is a major cause of bovine mastitis in dairy herds in China (Bi *et al*., 2016). According to the epidemiological investigation of bovine mastitis samples from different provinces of eastern China, most of *S. agalactiae* isolates were serotype *Ia* (89.2%) and serotype *II* (10.8%) (Yang *et al*., 2013). All isolates from Beijing, Hebei, and Inner Mongolia in northern China were belonging to serotype *Ia* (Du *et al*., 2016). However, epidemiological data remained incomplete for *S. agalactiae*, associated with bovine mastitis as data from large dairy herds from rest of provinces are lacking. Therefore, this study was designed to support the molecular epidemiology of *S. agalactiae* isolates from mastitis in large dairy herds of China using capsular serotyping and multilocus sequence typing (MLST) and antibiotic resistance assay.

MATERIALS AND METHODS

Ethics statement: The study was approved by the ethical committee of College of Animal Science and Technology, Shihezi University, China and the Institute of Veterinary Medicine, Xinjiang Academy of Animal Sciences, Urumqi, China. Milk samples were collected according to standard protocols and without any harm to the cattle.

Field milk samples, isolates and identification of *Streptococcus agalactiae*: The study was carried out between 2014 and 2017. A total of 2,225 milk samples were collected by standard sample collection method as recommended by National Mastitis Council (NMC, 1999). Sample were collected from 21 dairy herds in 10 provinces including Hainan (110), Henan (113), Liaoning (136), Inner Mongolia (489), Shaanxi (54), Shanxi (102), Shandong (185), Sichuan (165), Xinjiang (696), and Zhejiang (175) provinces of China (Fig. 1). Samples of 5-10 mL of milk were aseptically collected and transported to the laboratory within 24 - 48 h on ice packs, and then were tested for subclinical bovine mastitis using the Lanzhou Mastitis Test (LMT) kit (Lanzhou Veterinary Research Institute of China Academy of Agriculture Sciences, Lanzhou, China). LMT positive samples were subsequently used for *S. agalactiae* isolation. About 40 µL of LMT positive milk samples were inoculated on modified Granada medium and cultured at 37ºC for 24-48 h. Single pure orange colonies were inoculated in tryptic soy broth (BD Biosciences, New Jersey, USA) in triplicate for culture enrichment. Bacterial genomic DNA was extracted using the TIANamp Bacteria DNA kit (Tiangen Biotech, Beijing, China). Finally, the identification of *S. agalactiae* was performed by Polymerase Chain Reaction (PCR) according to the protocol and primers described by Mahmmod *et al.* (2015).

Detection of capsular serotyping: The capsular serotype was determined as described by Imperi *et al*. (2010). The PCR amplification conditions were: 95ºC for 5 min; 15 cycles of 95ºC for 60 s, 54ºC for 60 s, 72ºC for 2 min; 25 cycles of 95ºC for 60 s, 56ºC for 60 s, 72ºC for 2 min; extension of 72ºC for 10 min.

Multilocus Sequence Typing (MLST): Molecular epidemiology was also performed by MLST according to the methods of pubmlst (http://pubmlst.org/databases/). The sequences of seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkt*) were used to design primers to amplify the target gene fragments (primers are listed in Table 1). The PCR reaction was performed as: 95ºC for 5 min; 35 cycles of 95ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min and final extension at 72ºC for 10 min. The PCR products were then sequenced (Sanger sequencing, Sangon Biotech, Shanghai, China). The minimum spanning tree was performed using the BioNumerics 7.6.1 software (Applied Maths).

Table 1: Primers for MLST genotyping in this study

Primer	Sequence (5'→3')	Length (bp)
adhP-F	GTTGGTCATGGTGAAGCACT	672
adhP-R	ACTGTACCTCCAGCACGAAC	
pheS-F	GATTAAGGAGTAGTGGCACG	723
pheS-R	TTGAGATCGCCCATTGAAAT	
atr-F	CGATTCTCTCAGCTTTGTTA	627
atr-R	AAGAAATCTCTTGTGCGGAT	
glnA-F	CCGGCTACAGATGAACAATT	589
glnA-R	CTGATAATTGCCATTCCACG	
sdhA-F	AGAGCAAGCTAATAGCCAAC	646
sdhA-R	ATATCAGCAGCAACAAGTGC	
glcK-F	CTCGGAGGAACGACCATTAA	607
glcK-R	CTTGTAACAGTATCACCGTT	
tkt-F	CCAGGCTTTGATTTAGTTGA	859
tkt-R	AATAGCTTGTTGGCTTGAAA	

Virulence gene detection: The samples were next screened for the presence of six virulence genes, *hemolysin III*, *C-β protein*, *C-α protein*, *surface protein rib*, *hyaluronate lyase*, and *C5a peptidase*, as described by Sukhnanand *et al.* (2005) and Ayman *et al.* (2015) (Table 2).

Antimicrobial resistant testing: *S. agalactiae* strains were tested for antimicrobial resistance against erythromycin (15 µg), clindamycin (2 µg), ampicillin (10 µg), cefepime (30 µg), vancomycin (30 µg), levofloxacin (5 µg), and chloramphenicol (30 µg) antimicrobial agents (Oxoid, Hampshire, UK) using the standard disk diffusion method according to the recommendations of Clinical & Laboratory Standards Institute (CLSI, 2015).

Statistical analysis: All statistical analyses were performed by using the software SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA). Confidence intervals (CI) were estimated using the binomial distribution. Fisher's exact test was used to compare antimicrobial resistance rates between antimicrobials. Statistical significance was considered at the 5% level.

RESULTS

Prevalence of subclinical mastitis caused by *Streptococcus agalactiae*: Overall, 946 (42.5%) milk samples were detected positive for subclinical mastitis from the total 2225 milk samples collected from 21 dairy herds of 10 provinces of China. A total of 133 (14.1%) *S. agalactiae* isolates were identified from the mastitic milk

samples (Table 3). The most frequently isolated province was Xinjiang (21.7%), followed by Shanxi (17.9%) and Sichuan (17.7%) as shown in Table 3.

Capsular serotyping: Results of PCR-based amplification and subsequent sequencing showed all *S. agalactiae* isolates (n=133) were only amplified two fragments of 272 bp and 688 bp, indicating these isolates were capsular serotype *Ia*. In other words, the dominant capsular serotype of *S. agalactiae* in Hainan, Henan, Inner Mongolia, Liaoning, Shaanxi, Shanxi, Shandong, Sichuan, Xinjiang, Zhejiang provinces was serotype *Ia*.

Multilocus sequence typing (MLST) genotyping: All the 133 *S. agalactiae* isolates were genotyped into three sequence types (ST). ST103 was the most prevalent genotype (88.7%; 118/133), followed by ST67 (7.5%; 10/133) and ST4 (3.8%; 5/133). ST103 isolates were recovered from nine provinces including 40.6% (54/133), 15% (20/133), 8.3% (11/133), 6.8% (9/133), 6.0% (8/133), 3.8% (5/155), 3.8% (5/155), 1.5% (2/133), and 3.0% (4/133) isolates from Xinjiang, Inner Mongolia, Sichuan, Shandong, Liaoning, Shaanxi, Shanxi, Zhejiang and Henan provinces, respectively. ST67 isolates were only isolated in Xinjiang (6%, 8/133) and Zhejiang (1.5%, 2/133) provinces, and ST4 isolates (3.8%, 5/155) were recovered from Hainan province (Fig. 2).

Virulence gene distribution: Five out of six virulence factors were detected including *hemolysin III*, *C-α protein*, *surface protein rib*, *hyaluronate lyase*, and *C5a peptidase*; however, no *C-β protein* gene was detected. Of these genes, *hyaluronate lyase* was detected in all the *S. agalactiae* isolates (100%; 133/133), *C-α protein* gene in 16.5% (22/133) isolates and *surface protein rib* in 13.5% (18/133) isolates. *Hemolysin III* and *C5a peptidase* were less frequently, with only 2.3% (3/133) and 3.8% (5/133) isolates, respectively (Table 4). In addition, 21.6% (30/133) *S. agalactiae* isolates carried two virulence genes and 6.8% (9/133) isolates carried three virulence genes. Others harbored only one virulence gene and no isolate carried four or more virulence genes (Table 4).

Characterization of multi-virulence genes carried isolates: Overall, 39 *S. agalactiae* isolates were detected that carried two or three virulence genes. The isolates only with the *C-α protein/hyaluronate lyase* genes were found in Hainan (*n*=1), Henan (*n*=2), Liaoning (*n*=1), Inner Mongolia (*n*=1), Shanxi (*n*=1), Xinjiang (*n*=6) and Zhejiang (*n*=1), and distributed in ST103 (*n*=9), ST67 (*n*=3) and ST4 (*n*=1) as shown in Table 5. The isolates (*n*=12) that only carried the *surface protein rib/hyaluronate lyase* genes were all belonging to ST103 and were isolated in Liaoning (*n*=1), Shaanxi (*n*=1), Shanxi (*n*=2), Shandong (*n*=2) and Xinjiang (*n*=6). The isolates that only carried the *hyaluronate lyase* and *C5a peptidase* gene were belonging to ST103 and were distributed in Shandong (*n*=2), Sichuan (*n*=1) and Xinjiang (*n*=2). The isolates with *C-α protein***,** *hyaluronate lyase and hemolysin III* were all related to ST4 (n=3) and isolated from Hainan. However, the isolates that were positive for *C-α protein***,** *hyaluronate lyase* and *surface protein rib* were all belonging to ST103 (*n*=6), and found in Liaoning (*n*=2), Shanxi (*n*=1), Shandong (*n*=1), and Xinjiang (*n*=2) as shown in Table 5.

Table 2: Primers for virulence genes detection in this study

Primer	Protein	Sequence (5'→3')	Reference
*spb*1F	*hemolysin III*	GCTGAGACAGGGACAATTAC	Lin *et al*., 2011
*spb*1R		GTTGAAGGCAACTCAGTACC	
*bac*F	*C-β protein*	CTATTTTTGATATTGACAATGCAA	Lin *et al*., 2011
*bac*R		GTCGTTACTTCCTTGAGATGTAAC	
*bca*F	*C-α protein*	TAACAGTTATGATACTTCACAGAC	Lin *et al*., 2011
*bca*R		ACGACTTTCTTCCGTCCACTTAGG	
*rib*F	*surface protein rib*	CAGGAAGTGCTGTTACGTTAAAC	Lin *et al*., 2011
*rib*R		CGTCCCATTTAGGGTTCTTCC	
hyl-F	*hyaluronate lyase*	TTAACAAAGATATATAACAA	Ayman *et al.* 2015
hyl-R		TTTTAGAGAATGAGAAAAAA	
cfb-F	*CAMP factor*	CAAAGATAATGTTCAGGGAACAGATTATG	Ayman *et al*., 2015
cfb-R		CTTTTGTTCTAATGCCTTTACGTT	
cylE-F	*β-hemolysin/cytolysin*	TGACATTTACAAGTGACGAAG	Ayman *et al*., 2015
cylE-R		TTGCCAGGAGGAGAATAGGA	
scpB-F	*C5a peptidase*	ACAACGGAAGGCGCTACTGTTC	Ayman *et al*., 2015
scpB-R		ACCTGGTGTTTGACCTGAACT	

Table 3: Milk sample characterization

Provinces	Samples, *n*	Herds, *n*	Subclinical mastitis		*Streptococcus agalactiae*	
			n	% (95%CI) *	n	% (95%CI) ¶
Hainan	110	1	62	56.4 (47.1-65.6)	5	8.1 (1.3-14.8)
Henan	113	1	39	34.5 (25.7-43.3)	4	10.3 (0.7-19.8)
Liaoning	136	2	72	52.9 (44.6-62.3)	8	11.1 (3.9-18.4)
Inner Mongolia	489	3	200	40.9 (36.5-45.3)	20	10.0 (5.8-14.2)
Shaanxi	54	3	47	87.0 (78.1-96.0)	5	10.6 (1.8-19.5)
Shanxi	102	2	28	27.5 (18.8-36.1)	5	17.9 (3.7-32.0)
Shandong	185	2	92	49.7 (42.5-56.9)	9	9.8 (3.7-15.9)
Sichuan	165	1	62	37.6 (30.2-45.0)	11	17.7 (8.2-27.3)
Xinjiang	696	5	286	41.1 (37.4-44.7)	62	21.7 (16.9-26.5)
Zhejiang	175	1	58	33.1 (26.2-40.1)	4	6.9 (0.04-13.4)
Total	2225	21	946	42.5 (40.5-44.6)	133	14.1 (11.8-16.3)

Abbreviations: CI, confidence interval; *the detection rate of subclinical mastitis= number of subclinical mastitis milks / number of total milks collected; ¶the isolation rate of *Streptococcus agalactiae* = number of *Streptococcus agalactiae* / number of subclinical mastitis milks.

Fig. 1: The geographical distribution of milk samples collected in China. The blue-marked provinces indicate where the samples were collected.

Table 4: Virulence genes detected in *Streptococcus agalactiae* isolates

Variable	Isolates, n	Ratio, %(*n*/*N*)
Virulence genes		
C-β protein (*bac*)	0	0 (0/133)
C-α protein (*bca*)	22	16.5 (22/133)
hyaluronate lyase (*hyl*)	133	100.0 (133/133)
C5a peptidase (*scpB*)	5	3.8 (5/133)
hemolysin III (*spb1*)	3	2.3 (3/133)
surface protein rib (*rib*)	18	13.5 (18/133)
Two genes only		
bca/ hyl	13	9.8 (13/133)
hyl/rib	12	9.0 (12/133)
hyl/scpB	5	3.8 (5/133)
Three genes only		
bca/ hyl/spb1	3	2.3 (3/133)
bca/hyl/rib	6	4.5 (6/133)

Abbreviations: *bac*, *C-β protein*; *bca*, *C-α protein*; *hyl*, *hyaluronate lyase*; *scpB*, *C5a peptidase*; *spb1*, *hemolysin III*; *rib*, *surface protein rib*.

Table 5: The characterization of multi-virulence genes in isolates

Variable	2 genes			3 genes	
	bca/hyl (*n*=13)	*hyl/rib* (*n*=12)	*hyl/scpB* (*n*=5)	*bca/hyl/spb1* (*n*=3)	*bca/hyl/rib* (*n*=6)
STs					
ST4	1	0	0	3	0
ST67	3	0	0	0	0
ST103	9	12	5	0	6
Provinces					
Hainan	1	0	0	3	0
Henan	2	0	0	0	0
Liaoning	1	1	0	0	2
Inner mongolia	1	0	0	0	0
Shaanxi	0	1	0	0	0
Shanxi	1	2	0	0	1
Shandong	0	2	2	0	1
Sichuan	0	0	1	0	0
Xinjiang	6	6	2	0	2
Zhejiang	1	0	0	0	0

Abbreviations. *bac*, *C-β protein*, *bca*, *C-α protein*, *hyl*, *hyaluronate lyase*, *scpB*, *C5a peptidase*, *spb1*, *hemolysin III*, *rib*, *surface protein rib*.

Antimicrobial resistant test: The results of antimicrobial resistant test of *S. agalactiae* isolates are shown in Table 6. Most of the isolates were resistant to levofloxacin (63.9%; 85/133), followed by cefepime (3.8%, 5/133), chloramphenicol (2.3%, 3/133), ampicillin (2.3%, 3/133) and vancomycin (0.8%, 1/133), However, all *S. agalactiae* isolates were susceptible to erythromycin and clindamycin. Furthermore, two isolates (1.5%) exhibited multidrug resistance to ampicillin/cefotaxime/ levofloxacin and one isolate (0.8%) was resistant to ampicillin/cefotaxime/ levofloxacin/vancomycin (Table 6).

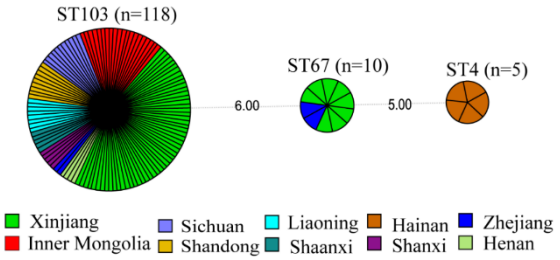

Fig. 2: Clustering of 3 STs of *Streptococcus agalactiae* isolates by use of minimum spanning tree. Each circle represents an ST, and the type number is indicated beside the circle. Colors indicate isolation sources. Circular division represents the number of different loci between two STs.

Table 6: Antimicrobial resistance of *Streptococcus agalactiae* isolates

Variable	Isolates, n	Ratio, % (*n*/*N*)
Erythromycin		
Susceptible	129	97.0 (129/133)
Intermediate	4	3.0 (4/133)
Resistant	0	0 (0/133)
Clindamycin		
Susceptible	132	99.2 (132/133)
Intermediate	1	0.8 (1/133)
Resistant	0	0 (0/133)
Ampicillin		
Susceptible	130	97.7 (130/133)
Intermediate	0	0 (0/133)
Resistant	3	2.3 (3/133)
Cefepime		
Susceptible	128	96.2 (128/133)
Intermediate	0	0 (0/133)
Resistant	5	3.8 (5/133)
Vancomycin		
Susceptible	132	97.3 (132/133)
Intermediate	0	0 (0/133)
Resistant	1	0.8 (1/133)
Levofloxacin		
Susceptible	24	18.1 (24/133)
Intermediate	24	18.1 (24/133)
Resistant	85	63.9 (85/133)
Chloramphenicol		
Susceptible	127	95.5 (127/133)
Intermediate	3	2.3 (3/133)
Resistant	3	2.3 (3/133)
Multi-drug resistant		
Ampicillin/Cefepime/Levofloxacin	2	1.5 (2/133)
Ampicillin/Cefepime/Levofloxacin/ Vancomycin	1	0.8 (1/133)

Characterization of multidrug resistant isolates: The three identified multidrug resistant *S. agalactiae* isolates were all belonging to serotype *Ia* and genotype ST103. Of the two ampicillin/cefotaxime/levofloxacin resistant isolates, both carried the hyaluronate lyase gene, one carried the surface protein rib gene, and the other four virulence genes were absent. These isolates were from Xinjiang and Liaoning. The isolate that carried the hyaluronate lyase gene but not the surface protein rib gene was found in Xinjiang.

DISCUSSION

Bovine mastitis is the most prevalent disease with substantial economic losses in the dairy industry. It is a common infectious disease with significant impact on milk production (Heikkila *et al.*, 2018). *S. agalactiae* is one of the important causes of mastitis (Ali *et al.*, 2008; Bi *et al.*, 2016). Thus, molecular epidemiology and characterization of *S. agalactiae* causing bovine mastitis would be of great importance for mastitis control and

clinical treatment. In this study, prevalence rate of *S. agalactiae* in subclinical mastitis samples was 14.1%. Moreover, based on capsular serotyping and MLST, 100% (133/133) isolates were identified as capsular serotype *Ia*, and 88.7% isolates were belonging to ST103, clustered in CC103. The hyaluronate lyase gene was detected in all samples and 22.6% of *S. agalactiae* isolates carried at least two virulence genes. Finally, antimicrobial resistance for *S. agalactiae* isolates obtained from dairy farms in China was determined.

The serotypes of *S. agalactiae* are complex and diverse, with some host-tropism. This study was not in accordance with the studies conducted in USA, which reported serotypes III and/or II as the predominant serotypes of *S. agalactiae* (Dogan *et al*., 2005), but was similar to the reports published from the eastern and northern China (Yang *et al*., 2013; Du *et al*., 2016), This is suggesting that serotype *Ia* is the dominant serotypes *S. agalactiae* of dairy cows in China.

The use of MLST genotyping in the molecular epidemiology of *S. agalactiae* is globally accepted and widely used technique for the genotyping, which mainly relies on the amplification and sequencing of seven housekeeping genes to obtain the unique ST number of each isolates. Our results indicated that 133 isolates were mainly distributed in ST103, followed by ST67 and ST4, with ST103 as the dominant genotype, which is similar to previous study in China (Du *et al*., 2016). In this study, ST4 isolates were only found in Hainan Province, suggesting the possibility of unique isolates in dairy cows in Hainan compared to those observed in the mainland provinces of China.

Virulence genes are associated with pathogenicity of *S. agalactiae*. In this study, *hyaluronate lyase* gene was detected in all isolates, indicating that this virulence gene is relatively conserved. *Hemolysin III* is a pore-forming hemolysin that can lyse red blood cells (Spencer *et al*., 2019), *C5a peptidase* gene is a serine protease that binds to fibronectin and invades epithelial cells (Emaneini *et al*., 2016), *surface protein rib* gene is found in serotype III isolates and provides protective immunity and invasive infections in neonates (Emaneini *et al*., 2016), *C-α protein* promotes adhesion to epithelial cells (Emaneini *et al*., 2016). Our results revealed that *hemolysin III*, *C-α protein*, *surface protein rib* and *C5a peptidase* gene are relatively uncommon, indicating that these genes might become a target for pathogenicity and vaccine development of *S. agalactiae*. Emaneini *et al.* (2016) reported *C5a peptidase*, *hyaluronate lyase* and *C-α protein* in only human isolates in Iran, however, our results showed that these three virulence genes could also be present in *S. agalactiae* from cattle suffering bovine mastitis.

Antimicrobial resistance has always been challenging for clinical treatment of bovine mastitis (Zhang *et al*., 2018; Cheng *et al*., 2019). The 133 isolates in the current study showed the highest resistance rate to levofloxacin, which is different from a previous report by Tian *et al.* (2019). One vancomycin-resistant *S. agalactiae* was also identified, but this has been rarely reported for *S. agalactiae* from bovine mastitis. Thus, further detailed analysis of the genetic characteristics is needed to trace its possible origin of resistance determinates in this isolate.

Conclusions: This study suggests that *S. agalactiae* was the main mastitic pathogen in China. It was detected in 14.1% of subclinical mastitis milk samples. *S. agalactiae* strains were mainly belonging to Serotype *Ia* and genotype ST103. Furthermore, hyaluronate lyase gene was prevalent in all *S. agalactiae* and 22.6% isolates carried at least two virulence genes. Finally, antimicrobial resistance for *S. agalactiae* isolates obtained from dairy farms in China were very high for levofloxacin. These data provided a baseline information for elucidating genetic diversity, population structure and antimicrobial resistance profiles of *S. agalactiae* in Chinese milk samples.

Authors contribution: DFW and BJ designed and conceived the study. XYY, JJL, XXM, YRW, GT, YHJ, and JX performed the experiments. BH and XYY analyzed the data and wrote the article. BH and TA critically edited and revised the manuscript. All the authors read and approved the final version.

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