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J Vet Res 67, 161-167, 2023
DOI:10.2478/jvetres-2023-0027

Molecular characterisation and antimicrobial resistance of *Streptococcus agalactiae* isolates from dairy farms in China

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Received: December 23, 2022 *Accepted: April 25, 2023*

Abstract

Introduction: *Streptococcus agalactiae* (*S. agalactiae*) is a pathogen causing bovine mastitis that results in considerable economic losses in the livestock sector. To understand the distribution and drug resistance characteristics of *S. agalactiae* from dairy cow mastitis cases in China, multilocus sequence typing (MLST) was carried out and the serotypes and drug resistance characteristics of the bacteria in the region were analysed. **Material and Methods:** A total of 21 strains of bovine *S. agalactiae* were characterised based on MLST, molecular serotyping, antimicrobial susceptibility testing, and the presence of drug resistance genes. **Results:** The serotypes were mainly Ia and II, accounting for 47.6% and 42.9% of all serotypes, respectively. Five sequence types (STs) were identified through MLST. The ST103 and ST1878 strains were predominant, with rates of 52.4% and 28.6%, respectively. The latter is a novel, previously uncharacterised sequence type. More than 90% of *S. agalactiae* strains were susceptible to penicillin, oxacillin, cephalothin, ceftiofur, gentamicin, florfenicol and sulfamethoxazole. The bacteria showed high resistance to tetracycline (85.7%), clindamycin (52.1%) and erythromycin (47.6%). Resistant genes were detected by PCR, the result of which showed that 47.6%, 33.3% and 38.1% of isolates carried the *tet*(M), *tet*(O) and *erm*(B) genes, respectively. **Conclusion:** The results of this study indicate that *S. agalactiae* show a high level of antimicrobial resistance. It is necessary to monitor the pathogens of mastitis to prevent the transmission of these bacteria.

Keywords: *Streptococcus agalactiae*, serotype, molecular characterisation, antimicrobial resistance, drug resistance gene.

Introduction

Bovine mastitis is a problematic disease for cattle husbandry, which if not prevented or treated promptly, leads to a decline in milk yield and quality and results in significant economic losses. *Streptococcus agalactiae* is a Gram-positive coccus that can infect multiple hosts, including humans and cattle (16, 33). In bovines, *S. agalactiae* can be transmitted to healthy cows under unhygienic milking conditions, typically occurring when milk from infected cows contaminates communal utensils and instruments in a milking facility (9). In clinical practice, antimicrobial therapy is the main strategy for controlling *S. agalactiae* infection in dairy cows. Antimicrobial resistance has become a worldwide problem for both human and animal health, and, with the current over-use and overreliance on antibiotics in animal husbandry, *S. agalactiae* drug resistance is increasing. In China, a surveillance plan for drug resistance of animal-derived bacteria has been implemented nationwide for many years, and an unbroken chain of supervision policy has been

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adopted over the production, operation and use of antibacterial drugs. The comprehensive strategy to curb drug resistance has achieved positive results, but some common microbial drug resistance problems are still increasing. In a study in Sichuan, China, *S. agalactiae* isolates were up to 100% sensitive to aminoglycosides (kanamycin, gentamicin, neomycin and tobramycin), and the resistance rate to β-lactams (penicillin, amoxicillin, ceftazidime and piperacillin) was up to 98.1% (35). In another study, 19.3 and 0.7% of *S. agalactiae* isolates were sensitive to tetracycline and daptomycin, respectively (26). Monitoring antimicrobial resistance trends is necessary to establish effective antimicrobial management in a dairy herd and to reduce the risk of further development and spread of drug resistance.

Serotyping is the traditional epidemiological tool for investigating *S. agalactiae* infections. Capsular polysaccharide (cps) is an important virulence factor in *S. agalactiae* and can be used for serotyping. *Streptococcus agalactiae* is classified into ten serotypes (Ia, Ib, and II to IX) depending on their capsular polysaccharide structures (6). The Ia and III serotypes are the most common in cows in China (15). Multilocus sequence typing (MLST) is a genotyping technique used to study the population structure of bacterial isolates from different geographic regions and is used to analyse the evolutionary relationships between pathogenic bacteria (14). Multiple *S. agalactiae* genotyping studies have been conducted in China, mainly focusing on pathogenic mechanisms and molecular typing in fish and humans. However, epidemiological studies on bovine *S. agalactiae* are limited.

The aim of this study was to investigate the antimicrobial resistance phenotypes, genotypes and epidemiological typing of *S. agalactiae* from dairy farms in China. The results of this study provide a theoretical basis for developing effective prevention and control measures against bovine mastitis.

Material and Methods

Sample collection. From April 2017 to December 2021, 21 strains of *S. agalactiae* were isolated from 535 milk samples from cows with clinical mastitis. The samples were collected from dairy farms in six provinces (autonomous regions) in China: Heilongjiang, Shanghai, Hebei, Shandong, Inner Mongolia and Xinjiang. Clinical mastitis was determined through visual examination by veterinarians and involved examining and palpating the udders and teats to detect heat, pain or swelling, and then checking milk secretion (colour and consistency).

Bacterial isolation and identification. Milk samples were collected under aseptic conditions from cows affected by clinical mastitis and immediately transported to the laboratory at 4°C. Laboratory tests were conducted within 24 h. The isolates were cultured in 3 mL of tryptic soy broth (Land Bridge Technology Co., Ltd., Beijing, China) at 37°C for 24 h. Genomic DNA was extracted according to the manufacturer's instructions (Tiangen BioTech Co., Ltd., Beijing, China). The extracted genomic DNA samples were stored at −20°C until use. The isolates were cultivated on Columbia agar base (Comagal Microbial Technology Co., Ltd., Shanghai, China) enriched with 5% sheep's blood and incubated at 37°C for 18–24 h. The cultures were checked for colony growth and morphological characteristics. Single colonies suspected to be *S. agalactiae* were isolated and confirmed using PCR and specific primers as previously described (5). A total of 21 *S. agalactiae* isolates were identified and stored at the Agricultural Quality and Safety Laboratory of Xinjiang. Supplementary Table S1 provides an overview of the strains used in this study.

Molecular serotyping. Molecular serotyping of *S. agalactiae* was performed using a multiplex PCR as previously described (22). Briefly, each 50 μL PCR reaction contained 2× RAPA 3G Multiplex PCR Mix (Ze Ye BioTech Co., Ltd., Shanghai, China), 400 nmol/L cpsI-Ia-6-7-F and cpsI-7-9-F primers, and 100 ng of bacterial genomic DNA template. The tubes were placed in a thermal cycler and the contents were amplified under the conditions as follows: 95°C for 5 min; followed by 15 cycles of 95°C for 60 s, 54°C for 60 s and 72°C for 2 min; followed next by 95°C for 60 s, 56°C for 60 s, and 72°C for 2 min; and a final cycle at 72°C for 10 min. The amplified products were electrophoresed on a 1% agarose gel with 0.5× tris, acetic acid and ethylenediaminetetraacetic acid buffer. Gels were visualised and photographed under ultraviolet illumination. Samples that could not be serotyped by this PCR method were classified as non-typeable (NT) strains.

Multilocus sequence typing. Multilocus sequence typing was performed as previously described (23). Seven different housekeeping genes were amplified by PCR and sequenced. Sequencing was completed by the Beijing Genome Institute (Beijing, China). The sequencing results were uploaded to the pubMLST database (https://pubmlst.org/sagalactiae/) for alignment and analysis and sequence types of individual strains were obtained. New alleles were submitted to the pubMLST database curator and allocated allele numbers and sequence types.

Antimicrobial susceptibility testing. Susceptibility of the isolates to 16 antibiotics was tested using a broth dilution method, following the Clinical and Laboratory Standards Institute instructions (8) for evaluating the minimum inhibitory concentration (MIC). *Streptococcus pneumoniae* ATCC 49619 was used as the quality control strain. The following antibiotics were tested: penicillin, ampicillin, clindamycin and ciprofloxacin (0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 μg/mL); erythromycin, cephalothin, ceftiofur, oxacillin, tetracycline, doxycycline, gentamicin and florfenicol (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128 μg/mL); sulfisoxazole (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1,024 μg/mL); kanamycin (0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 μg/mL); amoxicillin/clavulanic acid (0.25/0.12, 0.5/0.25, 1/0.5, 2/1, 4/2, 8/4, 16/8, 32/16, 64/32 and 128/64 μg/mL); and sulfamethoxazole

(0.12/2.4, 0.25/4.8, 0.5/9.5, 1/19, 2/38, 4/76, 8/152, 16/304, 32/608 and 64/1216 μg/mL). Minimum inhibitory concentrations were measured and the isolates classified as susceptible, intermediate, or resistant. The MIC_{50} and MIC_{90} values represent the concentrations of antimicrobial agents in which 50% and 90% of the strains were inhibited in growth, respectively.

Detection of drug resistance genes. The primer sequences of drug resistance genes were synthesised by Beijing Genome Institute (Beijing, China). Based on the results of testing the resistance of *S. agalactiae* phenotypically to antibiotics, eight drug resistance genes were selected for testing. Antimicrobial resistance genes were detected by PCR to tetracyclines: *tet*(M), *tet*(O), *tet*(L) and *tet*(S); macrolides: *erm*(A), *erm*(B), *mef*(A); and lincosamides: *lnu*(B). The sequences of primers used to target genes in the PCR analysis are shown in Table 1. The PCR system (total volume 25 μL) contained 50 ng of DNA template, 12.5 μL of 2× PCR Master Mix (Bioteke BioTech Co., Ltd., Beijing, China), 1 μL of forward and reverse primers (20 μm), and 8.5 μL of sterilised ultrapure water. The PCR amplification conditions were as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 45 s, annealing at different temperatures (Table 1) for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min. Electrophoresis and visualisation of PCR products were performed as described above. Correlations between resistance phenotypes and drug resistance genes were analysed.

Results

Molecular serotyping. The 21 *S. agalactiae* isolates were serotyped using multiplex PCR, and 10 type Ia strains (47.6% – the most prevalent), 9 type II strains (42.9% – the next most prevalent), 1 type III strain (4.8%), and 1 NT (4.8%) strain were identified.

Multilocus sequence typing. Allele numbers were obtained by aligning the sequences of the seven housekeeping genes against the pubMLST database. The seven allele numbers were combined to obtain the sequence types of the strains. The MLST results are shown in Table 2. A total of five sequence types were detected among the 21 *S. agalactiae* strains. Sequence type 103 was the most dominant (52.4%), followed by ST1878 (28.6%). Other sequence types included ST312 (9.5%), ST67 (4.8%) and ST309 (4.8%). The 1878 sequence type is a novel sequence based on a new allele (glck204) that has not been reported before and has a single locus variant (SLV) in ST67.

Antimicrobial susceptibility testing. The susceptibility of the 21 *S. agalactiae* isolates to 16 antibiotics was determined (Table 3). The percentages of the 21 *S. agalactiae* isolates resistant to tetracycline, clindamycin and erythromycin were 85.7, 52.1 and 47.6, respectively. Tetracycline had the highest MIC_{50} and MIC_{90} values (64 and 128 μg/mL, respectively). However, resistance to ampicillin, amoxicillin/clavulanic acid, gentamicin, florfenicol, kanamycin, ciprofloxacin, sulfisoxazole and sulfamethoxazole was shown by less than 30% of the isolates. The *S. agalactiae* strains were highly susceptible to penicillin, oxacillin, cephalothin, ceftiofur, gentamicin, florfenicol and sulfamethoxazole, yielding rates of 100%, 90.5%, 90.5%, 95.2%, 95.2% and 95.2%, respectively.

Detection of drug resistance gene. Eight drug resistance genes were examined in the 21 *S. agalactiae* strains following the antimicrobial drug test. Three of those for tetracycline resistance, *tet*(M), *tet*(O) and *tet*(S), were detected in 47.6%, 33.3% and 9.5% of the isolates, respectively. One macrolide resistance gene, *erm*(B), was detected in 38.1% of the isolates. The final four drug resistance genes, *tet*(L), *erm*(A), *mef*(A) and *lnu*(B) were not detected. The highest consistency rate between resistance phenotype and carriage of genetic markers of resistance was 80% and was for macrolide resistance, followed by 55.6% for tetracycline resistance (Table 4). Although clindamycin resistance was detected in 52.1% of the isolates, the lincosamide resistance gene *lnu*(B) was not detected.

Table 1. Primer sequence of drug resistance gene of *Streptococcus agalactiae*

Resistance gene	Primer sequence (5′–3′)	Annealing temperature (°C)	PCR product size (base pairs)	Reference
tet(M)	F: GTGGAGTACTACTACATTTACGAG R: GAAGCGGATCACTATCTGAG	55	359	31
tet(O)	F: GCGGAACATTGCATTTGAGGG R: CTCTATGGACAACCCGACAGAAG	51	538	
tet(L)	F: GGATCGATAGTAGCCATGGG R: GTATCCCACCAATGTAGCCG	53	516	
tet(S)	F: CGCTACATTTGCGAGACTCAG R: GGCTCTCATACTGAATGCCAC	55	569	
erm(A)	F: TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAGT	52	645	
erm(B)	F: GAAAAGGTACTCAACCAAATA R: CAGTAACGGTACTTAAATTGTTTAC	52	639	27
mef(A)	F: CGTAGCATTGGAACAGC R: TGCCGTAGTACAGCCAT	50	316	
lnu(B)	F: CCTACCTATTGTTTGTGGAA R: ATAACGTTACTCTCCTATTC	57	944	4

Table 2. Serotyping and sequence types in 21 *Streptococcus agalactiae* strains

ST	Allelic profile							Number of isolates in MLST (%)	Serotypes (Number of isolates)
	adhP	*pheS*	*atr*	*glnA*	*sdhA*	*glcK*	*tkt*		
103	16	1	6	2	9	9	2	11 (52.4%)	Ia (10) NT (1)
312	13	1	1	13	1	9	5	2 (9.5%)	II (2)
67	13	1	1	13	1	1	5	1 (4.8%)	II (1)
309	13	1	1	2	1	9	1	1 (4.8%)	III (1)
1878	13	1	1	13	1	204	5	6 (28.6%)	II (6)

MLST – multilocus sequence typing; NT – not typeable

Table 3. Antimicrobial resistance of *Streptococcus agalactiae* isolates

Antimicrobial	Phenotypic isolates (n = 21)			MIC (μg/mL)		
	Susceptible	Intermediate	Resistant	Range	MIC_{50}	MIC_{90}
penicillin	21 (100%)	0	0	≤0.13–>64.00	0.13	0.25
ampicillin	15 (71.4%)	0	6 (28.6%)	≤0.13–>64.00	0.25	1.00
amoxicillin/clavulanic acid	18 (85.7%)	1 (4.8%)	2 (9.5%)	≤0.25/0.12–>128.00/64.00	0.25/0.12	0.50/0.25
oxacillin	19 (90.5%)	0	2 (9.5%)	≤0.25–>128.00	0.50	1.00
cephalothin	19 (90.5%)	0	2 (9.5%)	≤0.25–>128.00	0.25	2.00
ceftiofur	20 (95.2%)	0	1 (4.8%)	≤0.25~>128.00	0.25	0.50
erythromycin	8 (38.1%)	3 (14.3%)	10 (47.6%)	≤0.25–>128.00	4.00	128.00
clindamycin	8 (38.1%)	1 (4.8%)	12 (52.1%)	≤0.13–>64.00	32.00	64.00
gentamicin	20 (95.2%)	0	1 (4.8%)	≤0.25–>128.00	1.00	4.00
doxycycline	6 (28.6%)	8 (38.1%)	7 (33.3%)	≤0.25–>128.00	8.00	16.00
florfenicol	20 (95.2%)	0	1 (4.8%)	≤0.25–>128.00	2.00	4.00
tetracycline	3 (14.3%)	0	18 (85.7%)	≤0.25–>128.00	64.00	128.00
kanamycin	14 (66.7%)	4 (19.0%)	3 (14.3%)	≤0.50–>256.00	16.00	64.00
ciprofloxacin	14 (66.7%)	3 (14.3%)	4 (19.0%)	≤0.13–>64.00	1.00	16.00
sulfisoxazole	15 (71.4%)	0	6 (28.6%)	≤2.00–>1024.00	4.00	1024.00
sulfamethoxazole	20 (95.2%)	0	1 (4.8%)	≤0.12/2.40–>64.00/1216.00	0.25/4.80	0.50/9.50

MIC_{50}/MIC_{90} – minimum inhibitory concentration of antimicrobial agents in which 50%/90% of the strains were inhibited in growth

Table 4. The correlations between resistance phenotypes and drug resistance genes of *Streptococcus agalactiae* isolates

Antimicrobial classes	Resistant gene	Number of strains with genotype	Number of phenotypes of resistance	Consistency rate (%)
tetracyclines	*tet*(M)	10	18	55.6%
	tet(O)	7		38.9%
	tet(L)	0		0
	tet(S)	2		11.1%
macrolides	*erm*(A)	0	10	0
	erm(B)	8		80.0%
	mef(A)	0		0
lincosamides	*lnu*(B)	0	12	0

Discussion

Streptococcus agalactiae is an important bacterial pathogen of bovine mastitis, which causes significant economic losses to dairy farms around the world. Understanding the variety of serotypes of the bacteria is helpful in designing measures to control the bacteria's spread and in supporting predictions about antimicrobial resistance. Molecular serotyping is often used to study the distribution and transmission characteristics of bacterial strains (7). Through serotyping, it is possible to gain an overall understanding of the characteristics of local epidemic strains and the speed and frequency of mutation. The Chinese epidemic picture of *S. agalactiae* is not a changeable one: the dominant serotype strains of the bacteria in China are relatively stable. In this study, serotype Ia was dominant (47.6%), and serotype II was the second most prevalent (42.9%). This result is

consistent with the epidemic serotype of bovine *S. agalactiae* in China established in a previous investigation (18). Studies have found a high correlation between *S. agalactiae* serotypes and sequence types. This study confirmed the correlation, because all ST 103 strains belonged to serotype Ia, and serotypes II and III had different sequence types.

Multilocus sequence typing was used to genotype the strains based on the allelic information of seven housekeeping genes, and was also used to further characterise the genetics and structure of the strain population (26, 37). An association between sequence types and host species or clinical manifestation was identified (26). The MLST results showed that 11 of the isolates were the ST103 type, accounting for the highest proportion (52.4%) of strains isolated in this study. In China, the frequency of ST103 isolates was relatively high, indicating that this is the main pathogenic strain in the country's different regions. Our data also confirmed the wide distribution of ST103. Recent studies have shown that ST103 is an environmental pathogen and is transmitted through environmental routes (2, 24). This subtype has previously been detected in the gastrointestinal tract of dairy cows and the water tank on the dairy farm. *Streptococcus agalactiae* is a more infectious mastitis pathogen than other *Streptococcus* spp. During milking, infectious strains can spread from one teat to another or from one cow to another in the same herd. Appropriate health measures should be taken to reduce the probability of infectious mastitis spread, and these measures need to reckon with *S. agalactiae* in particular.

Antibiotic therapy remains the primary strategy for treating mastitis in dairy cattle, but the widespread use of antibiotics in animal husbandry has led to an increase in antibiotic resistance in bacterial populations, and has affected human health along with the food chain. *Streptococcus agalactiae* has varying degrees of resistance to 16 antibiotics. In this study, its resistance rate to tetracycline was 85.7%, to clindamycin was 52.1%, and to erythromycin was 47.6%. These results are similar to those of previously published research results (11, 12). In almost all streptococcal mastitis, tetracycline resistance is the most common, followed by erythromycin resistance. Therefore, tetracycline is not recommended for the treatment of infections caused by *S. agalactiae*. A study showed a correlation between antimicrobial resistance and sequence type, finding the MIC of tetracycline against *S. agalactiae* ST103 to be higher than its MIC against other STs (26). In another study, all ST103 isolates from a farm resisted tetracycline (2). In this study, 10 out of 11 strains of *S. agalactiae* ST103 exhibited such resistance. Barros *et al.* (1) analysed the drug resistance rates of *S. agalactiae* to clindamycin and erythromycin over several years and saw the resistance of the isolates recovered after 2010 to the two antibiotics increase significantly. The average respective resistance rates to clindamycin and erythromycin were 6.5% and 6.8% before 2010 and 11.3% and 16.2% after 2010. These results highlight the importance of regular monitoring of *S. agalactiae* on dairy farms and testing it for antibiotic susceptibility.

Beta-lactam antibiotics such as penicillin, oxacillin and ampicillin are the most effective and widely used antibiotics in the treatment of mastitis caused by *Streptococcus* spp. (13, 25). Our results show that the susceptibility of *S. agalactiae* isolates to penicillin is 100%, which is similar to the high susceptibility of *S. agalactiae* isolates to β-lactam antibiotics reported in other studies (12, 34). The resistance of *S. agalactiae* to β-lactam antibiotics is usually low mainly because they cannot successfully obtain exogenous β-lactam resistance genes (30). Strains of the bacteria with reduced susceptibility to penicillin in routine antimicrobial susceptibility tests are still rarely reported. From these data, it is evident that β-lactam antibiotics should still be the first choice for the treatment of bovine mastitis caused by *S. agalactiae*, because these bacteria are susceptible to antibiotics of this class. During clinical treatment, several effective drugs should be selected for combined or alternate use, reducing the dosage or duration of use of a single drug in order to slow down the generation of drug-resistant strains.

Drug resistance genes confer resistance through mobile elements such as plasmids, transposons, integrons and phages (3). Tetracycline resistance is encoded by ribosome protection genes, including *tet*(M) and *tet*(O), or by the efflux pump gene *tet*(L) (19). The majority of *tet* genes are associated with either conjugative or mobile genetic elements and are broadly distributed across bacterial species. The strains of the *S. agalactiae* species generally resist tetracycline strongly, and *tet*(M) is a high frequency gene in pathogen sequence databases including *S. agalactiae* (28). In the present study, the prevalence of *tet*(M) (47.6%) and *tet*(O) (33.3%) genes in the tetracycline-resistant isolates was similar to that found by Duarte *et al.* (11). Conjugative transposons of the Tn916 family, which carry the *tet*(M) gene, are transmissible between different bacteria by a conjugative mechanism (32). Carriage of the *tet*(M) gene was 55.6% consistent with the tetracycline resistance phenotype; therefore, tetracycline resistance is mainly mediated by this gene.

Resistance to macrolides and lincosamide antibiotics is endowed by efflux pumps, ribosome modification, and drug inactivation (20). A methyltransferase encoded by the *erm* gene methylates ribosomes and is the predominant resistance mechanism against macrolide antibiotics in bacteria (10, 17). In this study, *erm*(B) was the gene with the second highest detection rate and it was found among the isolates with 38.1% prevalence, consistent with the findings reported by Hernandez *et al.* (21). Erythromycin resistance methylase, encoded by *erm* genes, may be the major mechanism of resistance in the present study. In our study, erythromycin resistance was mainly mediated by the *erm*(B) gene. The macrolide resistance phenotype was highly correlated with this resistance gene, reaching 80% coincidence. The *lnu*(B) gene encodes a lincosamide nucleotidyltransferase (29). While this gene marks

a resistance genotype, *in vitro* phenotypic resistance is not always accurately predicted by the genotype. The *lnu*(B) gene was not detected in clindamycin-resistant bacterial strains, contrary to what would be expected from susceptibility test results, possibly because other genes mediate clindamycin resistance in these strains. Gao *et al.* (15) found no resistance genes in some resistant isolates, indicating that the emergence or deletion of the corresponding drug resistance gene does not necessarily result in the bacteria having a corresponding drug-resistant or -susceptible phenotype.

The dominant pathogenic strain of bovine *S. agalactiae* identified in this study was ST103 of serotype Ia. Additionally, ST1878, a novel sequence type, was discovered and is reported here for the first time. This sequence type possesses a new *glc*K allele, *glc*K204. Although ST1878 isolates have not previously been described, the SLV of this sequence type, ST67, is the most common sequence type among bovine isolates. The latter sequence type is widely distributed on dairy farms in eastern China and Brazil (24, 36). The isolated strains showed high resistance to tetracycline, clindamycin and erythromycin. The drug resistance gene *tet*(M) was associated with tetracycline resistance, while erythromycin resistance was associated with the drug resistance gene *erm*(B). Analysis of *S. agalactiae* resistance, resistance genes and the mechanisms underlying genetic resistance in strains of this species discloses the operation of significant factors that guide the treatment of mastitis. This study provides a foundation for future studies aimed at clinical monitoring, prevention and control of mastitis in dairy cattle. Further epidemiological studies of *S. agalactiae* are needed to determine how the bacterial infection can best be controlled or prevented.

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Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This research was supported by the National Natural Science Foundation of China (32060797), China Post-doctoral project (282739), Tianshan Innovation Team (2022D14016), Xinjiang Science and Technology Assistance Project (2022E02012), Major Science and Technology Projects of the Xinjiang Uygur Autonomous Region (2022A020006), and Integration and Application of Key Technologies for Safe Production of High Quality Raw Milk (SR2020001).

Animal Rights Statement: None required.

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