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Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China

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ABSTRACT

Staphylococcus aureus is one of the main pathogens involved in dairy cow mastitis. Monitoring of antibiotic use would prove useful to assess the risk of *Staph. aureus* in raw milk. The objective of this work was to investigate the prevalence of *Staph. aureus* strains isolated from raw milk in northern China, and to characterize antimicrobial susceptibility of these strains and their key virulence genes. In total, 195 raw milk samples were collected from 195 dairy farms located in 4 cities of northern China from May to September 2015. Out of 195 samples, 54 (27.7%) were positive for *Staph. aureus*. Among these 54 samples, 54 strains of *Staph. aureus* were isolated, and 16 strains were identified as methicillin-resistant *Staph. aureus*. The strains exhibited high percentages of resistance to penicillin G (85.2%), ampicillin (79.6%), and erythromycin (46.3%). Moreover, 72% of the strains showed resistance to more than one antimicrobial agent. Overall, 63% of penicillin-resistant strains possessed the *blaZ* gene, and 60% of the erythromycin-resistant strains possessed *erm*(A), *erm*(B), *erm*(C), *msr*(A), or *msr*(B) genes with 8 different gene patterns. All isolates resistant to gentamicin, kanamycin, and oxacillin carried the *aac6'-aph2"*, *ant(4')-Ia*, and *mecA* genes, respectively. Two *tet*(M)-positive isolates carried specific genes of the Tn*916*-Tn*1545* transposon. The most predominant virulence genes were *sec*, *sea*, and *pvl*, which encode staphylococcal enterotoxins (*sec* and *sea*) and Panton-Valentine leukocidin, respectively. Thirty-two isolates (59.2%) harbored one or more virulence genes. The majority of *Staph. aureus* strains were multidrug resistant and carried multiple virulence genes, which may pose a risk to public health. Our data indicated that antimicrobial resistance of *Staph. aureus* was prevalent in dairy herds in northern China, and that antibiotics, especially penicillin G and ampicillin, to treat mastitis caused by *Staph. aureus* should be used with caution in northern China.

Key words: antimicrobial resistance, enterotoxins, northern China, raw milk, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is one of the main pathogens involved in dairy cow and goat mastitis worldwide, and it is an important opportunistic pathogen in raw milk (Cavicchioli et al., 2015; Wang et al., 2016). *Staphylococcus aureus* IMI results in significant economic losses due to reduced milk production and poor milk quality (Stapels et al., 2014), and *Staph. aureus* infections are related to the expression of virulence factors. Many virulence factors have been found in *Staph. aureus* isolated from bovine mastitis, including toxic shock syndrome toxin-1 (TSST-1), enterotoxins, enterotoxin-like exfoliative toxin, and Panton-Valentine leukocidin (Kot et al., 2016; Wang et al., 2016). These virulence factors are highly stable to heat or proteolytic enzymes and help bacteria to survive and multiply in the mammary gland (de Freitas Guimarães et al., 2013).

Antimicrobial therapy is an important tool in mastitis control programs, but *Staph. aureus* responds poorly to therapy with antimicrobial agents (Gomes and Henriques, 2016). *Staphylococcus aureus* is a pathogen with a remarkable ability to withstand antimicrobial agents and evade the human immune system. By recognizing the resistance mechanisms of *Staph. aureus*, effective measures in mastitis control programs can be established. Intrinsic resistance and acquired resistance have been shown to contribute to the ability of *Staph. aureus* to survive specific antimicrobial stress (Baym et al., 2016). It possesses numerous intrinsic factors that limit the effectiveness of specific antimicrobial agents (Rajagopal et al., 2016) and can develop acquired resistance to many other antimicrobial agents by carrying various

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resistance traits on plasmids or transposons (Chajecka-Wierzchowska et al., 2015). Acquired antimicrobial resistance has a transmission potential to humans (Ruegg et al., 2015). Therefore, monitoring antimicrobial resistance in *Staph. aureus* from raw milk is very important in order to predict the rate and type of antimicrobial resistance development and to make decisions regarding antibiotic treatments of animals from a food safety standpoint.

Studies of antimicrobial resistance and virulence traits in *Staph. aureus* have been performed in milk of bovines with mastitis in several countries, including Turkey, Brazil, Iran, and India, as well as the mideast of China (Gundogan and Avci, 2014; Cavicchioli et al., 2015; Jamali et al., 2015; Mistry et al., 2016; Zhang et al., 2016). However, reports on the incidence of antimicrobial resistance of *Staph. aureus* from raw milk in dairy herds in northern China are very limited. Continuous monitoring of antimicrobial resistance and virulence profiles of *Staph. aureus* could be useful to assess the risk of *Staph. aureus* in raw milk in northern China. Therefore, the objective of this work was to investigate the prevalence of *Staph. aureus* strains isolated from dairy herds in northern China from May to September 2015, and to characterize these strains by antimicrobial susceptibility and key virulence genes.

MATERIALS AND METHODS

Collection of Samples

A total of 195 raw milk samples were collected from 195 dairy farms located in major dairy-production cities of northern China (herd size ≥300, milking frequency 2–3 times per day, no clinical mastitis) from May to September 2015 (average daily temperature >20°C). We collected 75 samples from Hohhot, 50 from Beijing, 40 from Harbin, and 30 from Jinan (Figure 1). All samples were collected from the milk tank, transferred into sterile bottles, and transported immediately to laboratory at 4°C for bacteriological analysis.

Isolation and Identification of Staph. aureus

For isolation and detection of *Staph. aureus,* each sample (10 mL) was added to 90 mL of sterile peptone water and homogenized. The samples were placed onto Baird-Parker agar supplemented with 5% egg yolk and

Figure 1. Map of sampling locations. The 4 shaded regions are the main dairy-producing areas in northern China. In total, 75 samples were collected from Hohhot, 50 from Beijing, 40 from Harbin, and 30 from Jinan.

tellurite (Beijing Land Bridge Technology Ltd., Beijing, China). The plates were incubated for 24 to 48 h at 37°C. Colonies with typical black appearance and surrounded by a clear zone were enumerated as *Staph. aureus.*

Molecular Characterization of Staph. aureus

Presumptive colonies were confirmed by PCR detection (Bio-Rad S1000, Bio-Rad Laboratories, Hercules, CA) of the *Staph. aureus*-specific thermonuclease gene (*nuc*; Supplemental Table S1, https://doi.org/10.3168/jds.2017-13370). All isolates were stored at −80°C until use.

Genomic DNA of the *Staph. aureus* isolates was extracted using the InstaGene Matrix DNA extraction kit (Bio-Rad Laboratories) following the manufacturer's instructions. The PCR reactions were performed using the EmeraldAmp Max PCR Master Mix kit (Takara, Dalian, China) and followed the manufacturer's instructions. Briefly, 25-μL reactions were prepared, containing 12.5 μL of 2× EmeraldAmp Max PCR Master Mix kit (Takara), 1 μL of extracted DNA, 10 pmol of each primer, and ultrapure water (Takara). The amplification conditions were as follows: 94°C for 1 min; 37 cycles of 94°C for 1 min, 55°C for 30 s, and 72°C for 90 s; and 72°C for 3.5 min for a final extension step. Negative controls (without DNA template) and a positive control (*Staph. aureus* ATCC 6538 template) were included in all PCR assays.

Phenotypic Detection of Antimicrobial Resistance

Antimicrobial susceptibility patterns for recovered *Staph. aureus* isolates were determined by the agar disc diffusion method according to the guidelines of the Clinical Laboratory Standard Institute (Bauer et al., 1966; CLSI, 2012). Penicillin G (10U), amoxicillin-clavulanic acid (20/10 μg), tobramycin (10 μg), tetracycline (30 μg), gentamicin (10 μg), azithromycin (15 μg), oxacillin (1 μg), streptomycin (10 μg), cefoxitin (30 μg), oxytetracycline (30 μg), clindamycin (2 μg), erythromycin (15 μg), ampicillin (10 μg), chloramphenicol (30 μg), quinupristin-dalfopristin (15 μg), ciprofloxacin (5 μg), kanamycin (30 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg) were used as antimicrobial agents (Oxoid, Basingstoke, UK). *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 25922 were used as quality controls in each run. The experiment was repeated twice.

Antimicrobial Resistance Genes

Antimicrobial resistance genes to penicillin (*blaZ* and *mecA*), cefoxitin (*cfxA* and *mecA*), aminoglycoside (*aac6'-aph2"*, *ant(6)*-Ia, *aph3'*-IIIa), chloramphenicol (*fexA* and *catA*), tetracycline [*tet*(K), *tet*(L), *tet*(M) and *tet*(O)], erythromycin [*erm*(A), *erm*(B), *erm*(C), *msr*(A), and *msr*(B)], streptomycin [*ant(6)*-Ia], quinupristin-dalfopristin [*vga*(A) and *vga*(B)], and oxacillin (*mecA*) resistance genes, as well as the multidrug-resistance gene (*cfr*) were tested by PCR in all *Staph. aureus* strains (Supplemental Table S1https://doi.org/10.3168/jds.2017-13370).

Detection of Virulence Determinants

The genes encoding staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sek*, *sel*, *ser*, *ses*, *sej*, and *set*), enterotoxin-like (*selm*, *selo*, *selp*, and *selu*), toxic-shock syndrome toxin (*tst*-1), exfoliative toxin genes (*eta* and *etb*), and Panton-Valentine leukocidin (*pvl*) were detected by PCR. Amplified products were analyzed using 1.5% agarose gel electrophoresis and visualized by staining with SYBR Safe DNA Stain gel (Invitrogen, Carlsbad, CA). The information of all primers used for PCR is shown in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13370).

RESULTS

Prevalence of Staph. aureus

In the present study, 54 strains of *Staph. aureus* were isolated from raw milk, including 24 strains (32%) in 75 Hohhot samples, 8 strains (26.7%) in 30 Jinan samples, 11 strains (27.5%) in 40 Harbin samples, and 11 strains (22.0%) in 50 Beijing samples.

Antimicrobial Susceptibility Testing

The 54 isolates of *Staph. aureus* were tested for susceptibility to 18 antimicrobial agents by using the disc diffusion method. Antimicrobial resistance was most frequently observed to penicillin G (85.2%), followed by resistance to ampicillin (79.6%), erythromycin (46.3%), cefoxitin (42.6%), clindamycin (35.2%), azithromycin (35.2%), ciprofloxacin (29.6%), oxacillin (29.6%), sulfamethoxazole-trimethoprim (20.4%), kanamycin (14.8%), oxytetracycline (14.8%), tetracycline (13.0%), gentamicin (11.1%), streptomycin (9.26%), chloramphenicol (7.41%), tobramycin (5.56%), quinupristin-dalfopristin (3.7%), and amoxicillin-clavulanic acid (0%) (Table 1). Among the 54 *Staph. aureus* isolates, resistance to β-lactams (47.4%) was the most frequently observed, followed by resistance to macrolides (40.8%) and lincomycin (35.2%). Conversely, a low percentage of antimicrobial resistance was observed for chloramphenicol (7.41%) and streptogramin (3.7%).

Table 1. Antibiotic resistance of *Staphylococcus aureus* strains isolated from raw milk in northern China

Antibiotic class	Antibiotic	Strains, no. (%)
β-Lactams	Penicillin G	46 (85.2)
	Ampicillin	43 (79.6)
	Cefoxitin	23 (42.6)
	Oxacillin	16 (29.6)
	Amoxicillin-clavulanic acid	0 (0)
Macrolides	Erythromycin	25 (46.3)
	Azithromycin	19 (35.2)
Lincomycin	Clindamycin	19 (35.2)
Quinolones	Ciprofloxacin	16 (29.6)
Sulfonamides	Sulfamethoxazole-trimethoprim	11 (20.4)
Tetracyclines	Oxytetracycline	8 (14.8)
	Tetracycline	7 (13.0)
Aminoglycosides	Kanamycin	8 (14.8)
	Gentamicin	6 (11.1)
	Streptomycin	5 (9.26)
	Tobramycin	3 (5.56)
Streptogramin	Quinupristin-dalfopristin	2 (3.7)
Chloramphenicol	Chloramphenicol	4 (7.41)
Resistant to 1 antimicrobial agent		4 (7.4)
Resistant to 2 antimicrobial agents		6 (11.1)
Multi-drug resistant		33 (61.1)

Moreover, 72.2% of isolates showed resistance to 2 or more antibiotics. Two of the isolates were resistant to 12 of the 18 antibiotics tested, and 11 strains (20.4%) were susceptible to all tested antimicrobial agents. Sixteen isolates (29.6%) were identified as methicillin-resistant strains by antimicrobial susceptibility (oxacillin resistance). All methicillin-resistant *Staph. aureus* (**MRSA**) showed resistance (100%) to cefoxitin and penicillin.

Screening of Antimicrobial Resistance Genes

The results of antimicrobial resistance genes detection are presented in Table 2. All *Staph. aureus* isolates resistant to gentamicin, kanamycin, and oxacillin carried *aac6'-aph2"*, *ant(4')*-Ia, and *mecA* genes, respectively. The *blaZ*, *cfxA*, and *ant(4')*-Ia genes were detected in 63, 56.5, and 66.7% of penicillin-resistant, cefoxitin-resistant, and tobramycin-resistant isolates, respectively. The *tet*(K), *tet*(M), and *tet*(L) genes were found alone or together with others in the following percentages of the isolates: *tet*(K) (14.3%), *tet*(M) (42.9%), *tet*(K)+tet(L) (28.6%), and *tet*(M)+tet(L) (14.3%). The *tet*(L) gene was detected together with *tet*(K) and *tet*(M) in the same isolates. No tetracycline-resistant isolates harbored the *tet*(O) gene. Additionally, 2 *tet*(M)-positive strains carried a Tn*916*-Tn*1545* transposon gene. The erythromycin-resistant isolates (n = 25) harbored *erm*(B) (3 isolates, 12%), *erm*(C) (4 isolates, 16%), *msr*(A) (1 isolate, 4%), *erm*(B) and *erm*(C) (3 isolates, 12%), *erm*(C) and *erm(*A) (1 isolate, 4%), *erm*(C) and *msr*(A) (2 isolates, 8%), and *erm*(C) and *msr*(B) genes (1 isolate, 4%). However, *fexA* and *catA* genes were not detected in chloramphenicol-resistant strains. The *ant(6)*-Ia gene was not detected in streptomycin-resistant strains; and *vga*(A) and *vga*(B) genes were not detected in quinupristin-dalfopristin-resistant strains. Furthermore, the correlations between resistant phenotypes and genotypes for tetracycline, gentamicin, kanamycin, and oxacillin were 100%.

Distribution of Virulence Genes

As shown in Table 3, the most predominant toxin genes were *sec* (12/54, 22.2%), followed by *sea* (8/54, 14.8%), *pvl* (8/54, 14.8%), *tst* (8/54, 14.8%), *sej* (7/54, 13.0%), *selp* (6/54, 11.1%), *ses* (5/54, 9.3%), *seg* (5/54, 9.3%), *sei* (4/54, 7.4%), *sed* (4/54, 7.4%), *seb* (3/54, 5.6%), *ser* (1/54, 1.9%), *eta* (1/54, 1.9%), *see* (1/54, 1.9%), and *set* (1/54, 1.9%). The *seh*, *sek*, *sel*, *selm*, *selo*, *selu*, and *etb* genes were not detected in the *Staph. aureus* isolates.

As shown in Table 4, 59.2% of the strains (32/54) harbored one or more virulence genes, and 40.7% of the strains (22/54) did not detect any of the above virulence genes. Among the 32 *Staph. aureus* strains, one strain harbored 6 virulence genes together: *seg*, *sec*, *tst*, *sea*, *selp*, and *pvl* . Three isolates harbored 4 virulence genes with 2 different gene patterns, and the combination of *seg*, *sec*, *tst*, and *sea* was the dominant gene pattern. Eight isolates harbored 3 virulence genes with 8 different gene patterns. Nine isolates harbored 2 virulence genes with 7 different gene patterns, and the combinations of *sei* and *pvl* or *sei* and *sej* was the dominant gene pattern. Eleven isolates possessed one virulence gene with 7 different gene patterns.

Table 2. Antimicrobial resistance genes identified in *Staphylococcus aureus* strains isolated from raw milk samples from northern China

Antibiotic	Resistance gene	No. (%) of positive strains
Penicillin G	*blacZ*	29 (63)
	mecA	0
Cefoxitin	*cfxA*	13 (56.5)
	mecA	0
Tobramycin	*ant(4')*-Ia	2 (66.7)
Gentamicin	*aac6'-aph2"*	6 (100)
Chloramphenicol	*fexA*	0
	catA	0
Tetracycline	*tet*(K)	1 (14.3)
	tet(K) + *tet*(L)	2 (28.6)
	tet(M)	3 (42.9)
	tet(M) + *tet*(L)	1 (14.3)
	tet(O)	0
Erythromycin	*erm*(B)	3 (12)
	erm(B) + *erm*(C)	3 (12)
	erm(C)	4 (16)
	erm(C) + *erm*(A)	1 (4)
	erm(C) + *msr*(A)	2 (8)
	erm(C) + *msr*(B)	1 (4)
	msr(A)	1 (4)
	erm(A), *msr*(B), or both	0
Kanamycin	*ant(4')*-Ia	8 (100)
Oxacillin	*mecA*	16 (100)
Streptomycin	*ant(6)*-Ia	0
Quinupristin-dalfopristin	*vga*(A), *vga*(B), or both	0

DISCUSSION

In the present study, 25.8% (54/195) of raw milk samples were positive for *Staph. aureus* in raw milk. These results are significantly lower than in previous reports, which indicated that the occurrence rate of *Staph. aureus* in raw milk was 83% in Turkey (Bartolomeoli et al., 2009), 66.7% in Malaysia (Andre et al., 2008), 56% in Brazil (Gundogan and Avci, 2014), and 41.0% in Italy (Traversa et al., 2015). In contrast, a much lower occurrence rate (12.4%) of *Staph. aureus* was found in raw milk in Iran (Jamali et al., 2015). Overall, the results of our current study indicate that *Staph. aureus* is common in raw milk collected from dairy herds throughout northern China. Major sources of *Staph. aureus* in raw milk might include infected cows and inappropriate hygiene conditions (Jamali, et al., 2015). Further research is ongoing and will explore methods of controlling *Staph. aureus* occurrence in raw milk.

In our analysis, 72.2% of isolates showed resistance to 2 or more antibiotics, and 61.1% of *Staph. aureus*

Table 3. Virulence genes identified in *Staphylococcus aureus* strains isolated from raw milk samples from northern China (n = 54)

Class of toxin	Type of toxin	Gene	No. (%) of positive strains
Enterotoxins	Enterotoxin A	*sea*	8 (14.8)
	Enterotoxin B	*seb*	3 (5.6)
	Enterotoxin C	*sec*	12 (22.2)
	Enterotoxin D	*sed*	4 (7.4)
	Enterotoxin E	*see*	1 (1.9)
	Enterotoxin G	*seg*	5 (9.3)
	Enterotoxin I	*sei*	4 (7.4)
	Enterotoxin R	*ser*	1 (1.9)
	Enterotoxin S	*ses*	5 (9.3)
	Enterotoxin T	*set*	1 (1.9)
	Enterotoxin J	*sej*	7 (13.0)
Enterotoxin-like	Enterotoxin-like protein P	*selp*	6 (11.1)
Toxic-shock syndrome toxin	Toxic-shock syndrome toxin	*tst-1*	8 (14.8)
Exfoliative toxins	Exfoliative toxin A	*eta*	1 (1.9)
Panton-Valentine leukocidin	Panton-Valentine leukocidin	*pvl*	8 (14.8)

Table 4. Multiple gene patterns identified in *Staphylococcus aureus* strains isolated from raw milk samples from northern China

No. of toxin genes	Genotypes	No. of isolates	Total
6	*seg*, *sec*, *tst*, *sea*, *selp*, *pvl*	1	1
4	*sec*, *tst*, *sea*, *pvl*	1	3
	seg, *sec*, *tst*, *sea*	2	
3	*sec*, *eta*, *pvl*	1	8
	sed, *sei*, *pvl*	1	
	sed, *seg*, *sej*	1	
	see, *sec*, *sea*	1	
	seg, *sec*, *selp*	1	
	sej, *sec*, *selp*	1	
	sej, *selp*, *sea*	1	
	sec, *tst*, *sea*	1	
2	*sea*, *sei*	1	9
	sea, *sed*	1	
	seg, *tst*	1	
	sei, *sej*	2	
	sei, *pvl*	2	
	sei, *sec*	1	
	sei, *tst*	1	
1	*sec*	1	11
	sed	2	
	seg	1	
	sej	1	
	ser	2	
	seb	3	
	pvl	1	

isolates showed multidrug resistance (**MDR**), which was much higher than reported in other studies. Other researchers have reported lower rates of MDR *Staph. aureus* (Haran et al., 2012; Jamali et al., 2015). The high prevalence of drug-resistant *Staph. aureus* from raw milk—the raw material for dairy products—should be considered a potential risk to human health in northern China. Moreover, we investigated antibiotic use for mastitis in northern China in our previous survey, and found that 6 antibiotics (penicillin, ampicillin, ciprofloxacin, sulfamethoxazole-trimethoprim, gentamicin, and streptomycin) are commonly used in dairy mastitis therapy (data not shown). In the current study, most of the *Staph. aureus* strains showed a high percentage of antimicrobial resistance to penicillin, ampicillin, ciprofloxacin, and sulfamethoxazole-trimethoprim. The results indicated a correlation between antibiotic use and antimicrobial resistance. Similar findings were reported by Saini et al. (2012), who found that herd-level use of certain antimicrobials administered for mastitis treatment was positively associated with antimicrobial resistance in isolates from a clinical mastitis sample. Data on antibiotic susceptibility of *Staph. aureus* can be helpful in choosing the most suitable antibiotic for mastitis treatment (Wang et al., 2016). Based on the high percentage of resistance to penicillin and ampicillin, these antibiotics should be used with caution for mastitis caused by *Staph. aureus* in dairy herds in northern China.

Methicillin-resistant *Staph. aureus* is an emerging pathogen in livestock animals that is readily transferable to humans that are in contact with livestock. In this study, 16 isolates (29.6%) were identified as MRSA by antimicrobial susceptibility, and all MRSA isolates showed resistance to cefoxitin and penicillin, which is similar to findings of several prior studies (Bhargava and Zhang, 2012; Chajecka-Wierzchowska et al., 2015). The MRSA resistance was caused by the expression of a modified penicillin-binding protein (PBP), named PBP2A, with reduced affinity for β-lactam antimicrobial agents. For MRSA, β-lactams cannot bind to native PBP2A, so synthesis of peptidoglycan and bacterial growth occur normally (Diaz et al., 2016).

Tetracylines are broad-spectrum antimicrobial agents frequently used in the treatment of infections in cattle (Chajecka-Wierzchowska et al., 2016). In our study, 13.9% of *Staph. aureus* isolates showed resistance to tetracylines. The high prevalence of resistance to tetracycline has been reported previously among *Staph. aureus* isolates from different sources (Andre et al., 2008; Gundogan and Avci, 2014; Jamali et al., 2015). Moreover, tetracycline resistance can be conferred by genes encoding efflux proteins TetK and TetL or ribosomal protection proteins TetM, TetO, and TetS (Huys et al., 2004). In our study, all the tetracycline-resistant isolates harbored at least one tetracycline resistance determinant, of which *tet*(M) was most frequent. These results are in agreement with the findings of Bhargava

and Zhang (2012), who detected *tet*(M) in 64.3% of investigated strains. In the current study, *tet*(M)-positive isolates carried the transposon and *tet*(M) was the most prevalent *tet* gene detected among the tetracycline-resistant *Staph. aureus* isolates. Several studies have shown that the spread of resistance to antimicrobial is largely due to the acquisition of plasmids or transposons (Lopatkin et al., 2016; Pehrsson et al., 2016).

In this study, the correlation between resistant phenotypes and genotypes for tetracycline, gentamicin, kanamycin, and oxacillin was 100%. Genotypic prediction of resistance relies on highly curated databases of known resistance determinants and cannot identify new mechanisms. Therefore, testing phenotypic susceptibility is still necessary to fully account for resistance and predict new mechanisms of resistance (Zhao et al., 2015). Knowing which antimicrobial resistance genes are present in bacterial populations is critical to developing new strategies to combat antimicrobial resistance, including improving antimicrobial resistance and implementing surveillance programs for foodborne diseases.

Another important factor in the pathogenesis of *Staph. aureus* infections is virulence. Once enterotoxins are produced, they generally retain their biological activity even after heat treatment (Cavicchioli et al., 2015). In the present study, 19 staphylococcal enterotoxin genes were tested, and 60.3% of *Staph. aureus* isolates harbored one or more of these virulence genes. The enterotoxin gene distribution in *Staph. aureus* isolates from raw milk could provide epidemiological information for public health and food safety. The *sec*, *sea*, *pvl*, and *tst* genes were frequently detected in isolates from raw milk in our study. The most frequently reported virulence gene in *Staph. aureus* from goat milk is the *sec* gene (Cavicchioli et al., 2015); *tst* and *sec* are usually detected together from goat and ovine milk (Xing et al., 2016); and *pvl* is usually detected in *Staph. aureus* from raw milk. The *pvl* gene plays an important role in pathogenesis with a strong ability to stimulate cytolysis of neutrophils, apoptosis, and production of pro-inflammatory molecules (Liu, 2009). This suggests that attention should be paid not only to classical enterotoxins in raw milk and other dairy products, but also to other virulence factors detected among pathogenic staphylococci.

CONCLUSIONS

This study assessed the antimicrobial resistance of *Staph. aureus* strains isolated from raw milk in northern China. Our data indicate that *Staph. aureus* is prevalent in raw milk from herds in the northern region. The detected *Staph. aureus* strains exhibited a high percentage of antimicrobial resistant and carried multiple virulence genes. Therefore, it is important to monitor the use of antimicrobial agents and the potential transfer of antimicrobial resistance genes in northern China. Penicillin G and ampicillin for mastitis caused by *Staph. aureus* should be used with caution in dairy herds in northern China. Further studies should be carried out to evaluate the possibility of acquiring, transferring, and transmitting antimicrobial resistance genes among *Staph. aureus*.

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