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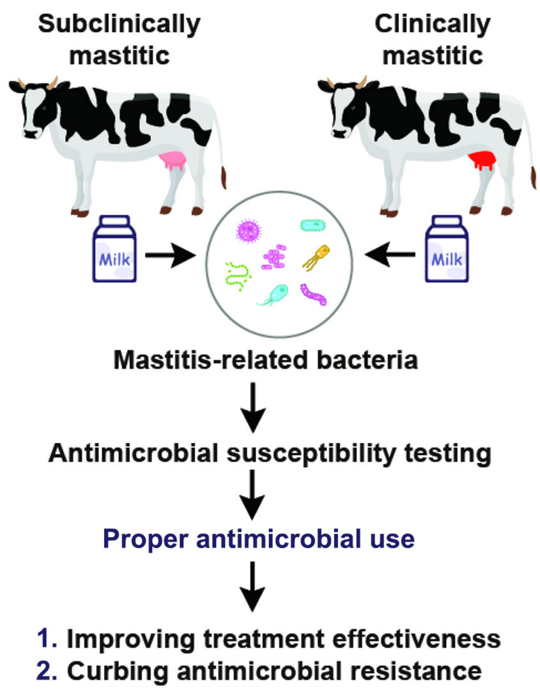
Short Communication
Dairy Foods

Antimicrobial resistance profiles of common mastitis pathogens on large Chinese dairy farms

Xiangbin Song,[1,2]* Yaxin Wang,[1]* Rina Bai,[1]* Xiaoyan Pei,[3] Hongyan Xu,[3] Kui Zhu,[1] and Congming Wu[1]†

Graphical Abstract

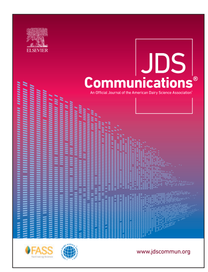


Summary

This study reveals the antimicrobial resistance (AMR) profiles of 7 types of predominant bovine mastitis pathogens on large Chinese dairy farms, including *Staphylococcus aureus* (97), coagulase-negative staphylococci (CNS, 352), *Streptococcus agalactiae* (38), non-agalactiae streptococci (50), *Acinetobacter* spp. (52), *Escherichia* spp. (43), and *Klebsiella* spp. (41). More than 75% of *Staph. aureus* and CNS showed resistance to penicillin (PEN). More than 30% of *Escherichia* spp. showed resistance to ampicillin. However, less than 10% of CNS and non-agalactiae streptococci showed resistance to amoxicillin/clavulanate (AMC), cephalexin (LEX), ceftiofur (EFT), and rifaximin (RIX); and less than 10% of *Staph. aureus*, *Strep. agalactiae*, and *Escherichia* spp. showed resistance to AMC, oxacillin, LEX, EFT, and RIX; PEN, AMC, LEX, EFT, and RIX; and AMC and EFT, respectively.

Highlights

- In Chinese dairy farms, CNS was the predominant bovine mastitis pathogen.
- More than 75% of *Staph. aureus* and CNS showed resistance to PEN.
- More than 30% of *Escherichia* spp. showed resistance to ampicillin.
- Less than 10% of CNS and non-agalactiae streptococci were resistant to AMC, LEX, EFT, and RIX.



[1]National Key Laboratory of Veterinary Public Health and Safety, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China, [2]Shandong Provincial Key Laboratory of Quality Safety Monitoring and Risk Assessment for Animal Products, Shandong Center for Quality Control of Feed and Veterinary Drug, Jinan 250100, PR China, [3]Yili Innovation Center, Inner Mongolia Yili Industrial Group Co. Ltd., Hohhot 010110, PR China. *These authors contributed equally to this work. †Corresponding author: wucm@cau.edu.cn. Received June 07, 2023. Accepted November 18, 2023.

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Abstract: The primary objective of this study was to determine the antimicrobial resistance (AMR) profile of common mastitis pathogens on large Chinese dairy farms. A total of 673 isolates, including *Staphylococcus aureus* (14.41%, 97/673), coagulase-negative staphylococci (CNS, 52.30%, 352/673), *Streptococcus agalactiae* (5.64%, 38/673), non-*agalactiae* streptococci (7.42%, 50/673), *Acinetobacter* spp. (7.72%, 52/673), *Escherichia* spp. (6.39%, 43/673), and *Klebsiella* spp. (6.09%, 41/673), were collected from 15 large Chinese dairy farms in 12 provinces. The AMR profiles were measured using a microdilution method. Our results showed that more than 75% of *Staph. aureus* (87/97) and CNS (291/352) were resistant to penicillin (PEN). More than 30% of *Escherichia* spp. (15/43) showed resistance to ampicillin (AMP). However, less than 10% CNS and non-*agalactiae* streptococci showed resistance to amoxicillin/clavulanate (AMC; 1/352; 0/50), cephalexin (LEX; 1/352; 0/50), ceftiofur (EFT; 10/352; 0/50), and rifaximin (RIX; 21/352; 2/50); less than 10% *Staph. aureus* showed resistance to AMC (1/97), oxacillin (OX; 3/97), LEX (1/97), EFT (2/97), and RIX (2/97); less than 10% *Strep. agalactiae* showed resistance to PEN (3/38), AMC (0/38), LEX (0/38), EFT (0/38), and RIX (0/38); and less than 10% *Escherichia* spp. showed resistance to AMC (1/43) and EFT (4/43). These results suggested that most mastitis pathogens were susceptible to most antimicrobials with exceptions of *Staph. aureus* tested against penicillin or ampicillin and CNS against penicillin or oxacillin. To control the AMR threat in Chinese dairy farms, a nationwide surveillance program for AMR of bovine mastitis pathogens is needed.

Mastitis is one of the most prevalent diseases in dairy cows worldwide, affecting animal well-being and dairy farm profitability (Ruegg, 2017). Over 200 different pathogens have been reported to be associated with bovine mastitis, and most of these pathogens are bacteria (Sharun et al., 2021). Intensive dairy farming has increased drastically in the past 3 decades in China, with more than 50% of dairy farms having a herd size of >500 cows (Wang et al., 2022). The most frequently isolated pathogens are *Staphylococcus* spp. and *Streptococcus* spp. in Chinese large dairy herds (Song et al., 2020). Moreover, *Escherichia coli* and *Klebsiella* spp. are also the predominant bovine mastitis pathogens in other countries (Schukken et al., 2012; Klaas and Zadoks, 2018).

The treatment of mastitis accounts for the majority of antimicrobial use in dairy farms (Kuipers et al., 2016; Ruegg, 2017). Societal concerns regarding the emergence of antimicrobial resistance (**AMR**) and consequential public health threats, such as interspecies transmission of AMR pathogens or genes between animals and humans due to the abuse of antimicrobials for animal use, have increased in recent years (Van Boeckel et al., 2019).

Monitoring AMR of mastitis pathogens is essential for prudent antimicrobial use, improving treatment effectiveness, and curbing the development of AMR (Ruegg, 2017). Prevalence of AMR of 5 predominant pathogens isolated from clinical mastitis (**CM**) has been recently reported in large Chinese dairy herds (Cheng et al., 2019). Moreover, prevalence of AMR of a certain pathogen isolated from CM and subclinical mastitis (**SCM**) have also been recently reported, including *Staphylococcus aureus* (Zhang et al., 2016; Liu et al., 2020) isolated from CM, *Streptococcus agalactiae* isolated from SCM (Lin et al., 2021) and CM (Liu et al., 2022), and *Streptococcus* spp. isolated from SCM and CM (Tian et al., 2019). However, there is still a lack of investigation of the prevalence of AMR on common pathogens associated with both CM and SCM in large Chinese dairy farms. Thus, this study aims to determine the AMR profiles of the predominant bacteria (*Staph. aureus*, CNS, *Strep. agalactiae*, non-*agalactiae* streptococci, *Acinetobacter* spp., *Escherichia* spp., and *Klebsiella* spp.) isolated from CM and SCM on large Chinese dairy farms and consequently provide evidence for antimicrobial use for bovine mastitis on large Chinese dairy farms.

A total of 673 isolates, including *Staph. aureus* (97), CNS (352), *Strep. agalactiae* (38), non-*agalactiae* streptococci (50), *Acinetobacter* spp. (52), *E. coli* (43), and *Klebsiella* spp. (41), were identified in mastitis milk samples collected from 15 large dairy farms in 12 provinces of China from July 2018 to October 2019. The CM and SCM were monitored daily at milking time. First, a case of CM was defined by clinical signs such as abnormal milk, abnormal udder (swollen, red, or hard), or fever. Second, cases without clinical signs were determined by the Lanzhou Mastitis Test (**LMT**); the LMT is a diagnostic technique of SCM homologous to the California Mastitis Test, where −, +, ++, and +++ correspond to negative, weakly positive, positive, and strongly positive, respectively (Liu et al., 1983). The milk samples were collected and processed according to guidelines from the National Mastitis Council (NMC, 2017) with slight modifications. The samples were collected in

[1]National Key Laboratory of Veterinary Public Health and Safety, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China, [2]Shandong Provincial Key Laboratory of Quality Safety Monitoring and Risk Assessment for Animal Products, Shandong Center for Quality Control of Feed and Veterinary Drug, Jinan 250100, PR China, [3]Yili Innovation Center, Inner Mongolia Yili Industrial Group Co. Ltd., Hohhot 010110, PR China. *These authors contributed equally to this work. †Corresponding author: wucm@cau.edu.cn. Received June 07, 2023. Accepted November 18, 2023.

sterile bottles (5–10 mL) and transported on ice to the mastitis diagnostic laboratory at China Agricultural University within 48 h.

After arriving at the laboratory, 10 μL of milk from each sample was plated on brain heart infusion agar (**BHIA**) with 5% defibrinated sheep blood, and cultured aerobically at 37°C for 24 to 48 h. If no *Staph. aureus* or *Strep. agalactiae* were isolated from a sample, then milk samples with >2 different species were considered contaminated (Gao et al., 2017). All bacteria isolated from mastitis milk samples were first identified by MALDI-TOF, and colonies that failed to be identified by MALDI-TOF were subjected to 16S rDNA sequencing. The 16S rDNA sequences were searched using BLAST against the National Center for Biotechnology Information database for identification. Subsequently, all isolates were stored at −80°C.

The bacterial isolates of *Streptococcus* spp. were recovered on BHIA with 5% defibrinated sheep blood and then cultured aerobically at 37°C overnight. Afterward, a single colony was transferred to Mueller-Hinton broth medium (**MHB**), with the exception of *Streptococcus* spp. Bovine serum (5%) was added to MHB to promote the growth of *Streptococcus* spp. We used a bacterial Densicheck turbidimeter (DENSIMAT, bioMérieux) to adjust the suspension to a density equivalent to a 0.5 McFarland standard for MIC determination.

The AMR profiles of all isolates were measured using a microdilution method for antimicrobials, following the instructions provided by the Clinical Laboratory and Standards Institute (**CLSI**) (CLSI, 2013, 2018) and the European Committee on Antimicrobial Susceptibility Testing (**EUCAST**, 2016). The tested antimicrobials are commonly used to treat bovine mastitis in practice in China. Eight antimicrobials with different concentrations for gram-positive bacteria species were used: penicillin (0.25–64 μg/mL), ampicillin (0.25–64 μg/mL), amoxicillin/clavulanate (**amoxi/clav**, 0.5/0.25–64/32 μg/mL), oxacillin (0.5–64 μg/mL), cefalexin (0.5–64 μg/mL), ceftiofur (0.5–64 μg/mL), lincomycin (0.25–64 μg/mL), and rifaximin (0.25–64 μg/mL). The oxacillin results were applied to cloxacillin (CLSI, 2018). For gram-negative isolates except *Klebsiella* spp., 4 antimicrobials were tested: ampicillin (0.25–128 μg/mL), amoxi/clav (0.25/0.125–128/64 μg/mL), ceftiofur (0.125–128 μg/mL), and rifaximin (0.25–128 μg/mL). Positive controls (only bacteria) and negative controls (free of both antimicrobials and bacteria) were also included in all plates. *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. In this study, all antimicrobials used were obtained from the China Institute of Veterinary Drug Control (Beijing, China). We determined the MIC_{50} and MIC_{90} values for each isolate-antimicrobial combination to determine the minimum concentration of an antimicrobial that inhibited the visible growth of ≥50% and ≥90% of bacteria, respectively. Notably, the MIC values between different antimicrobials could not be directly compared. For amoxi/clav, the MIC of the former agent was considered the MIC of the combination.

The AMR profile of bacterial isolates was defined according to CLSI (2013, 2018) or EUCAST (2016). In the absence of animals' breakpoints, CLSI breakpoints or EUCAST breakpoints were used in sequential order.

Staphylococcus aureus and CNS are important mastitis pathogens in dairy herds (Ruegg, 2017). The MIC of 449 *Staphylococcus* spp. isolates were assessed, and the MIC of all tested antimicrobials greatly varied. All MIC_{90} values were ≥1 dilution higher than the MIC_{50} values (Table 1). *Staphylococcus aureus* isolates had the highest resistance rate against penicillin (89.69%), followed by ampicillin (64.95%), lincomycin (24.74%), oxacillin (3.09%), ceftiofur (2.06%), rifaximin (2.06%), amoxi/clav (1.03%), and cefalexin (1.03%). For *Staph. aureus*, the MIC_{90} values of the 4 tested antimicrobials were ≤1 μg/mL. Resistance to penicillin was the highest (82.67%), followed by oxacillin (50.00%), ampicillin (35.80%), lincomycin (21.59%), rifaximin (5.97%), ceftiofur (2.84%), cefalexin (0.57%), and amoxi/clav (0.28%) for CNS. Notably, *Staph. aureus* isolates isolated from CM showed higher resistance rates to most antimicrobials than these isolated from SCM with exceptions of isolates tested against penicillin (Table 1). For CNS, the MIC_{90} values of the 6 tested antimicrobials were ≤1 μg/mL. Notably, CNS isolates isolated from CM showed higher resistance rates to most antimicrobials than these isolated from SCM with exceptions of isolates tested against oxacillin and rifaximin (Table 1). The *Staph. aureus* isolates were the most resistant to penicillin, which was in agreement with previous studies in China (Zhang et al., 2016). However, a relatively lower resistance (78.9%) of *Staph. aureus* to penicillin was observed in Brazil (Dorneles et al., 2019). We tested oxacillin rather than cloxacillin because it was more reliable for detecting methicillin-resistant isolates of *Staph. aureus* (**MRSA**; De Oliveira et al., 2000). Only 3.09% of *Staph. aureus* isolates were classified as MRSA, which agreed with findings in Sweden (Bengtsson et al., 2009); however, this value was lower than the amount estimated by Cheng et al. (2019) in China. However, the resistance rate against oxacillin was 50.00% in CNS isolates, which was inconsistent with a report in Europe (de Jong et al., 2018).

For decades, *Strep. agalactiae* has been an important contagious pathogen that causes bovine mastitis worldwide, resulting in considerable economic losses (Ruegg, 2017). In this study, the MIC test was performed on 38 isolates of *Strep. agalactiae* and 50 isolates of non-*agalactiae* streptococci (*Streptococcus dysgalactiae* and *Streptococcus uberis*; Table 2). The MIC distribution of *Strep. agalactiae* was wider than that of non-*agalactiae* streptococci. The resistances of *Strep. agalactiae* isolates were commonly observed against lincomycin (44.74%), ampicillin (36.84%), and penicillin (7.89%); however, no *Strep. agalactiae* isolates were resistant to amoxi/clav, cefalexin, ceftiofur, and rifaximin. Notably, *Strep. agalactiae* isolates isolated from CM showed higher resistance rates to most antimicrobials than these isolated from SCM with the exceptions of isolates tested against lincomycin (Table 2). Results similar to our study were also reported in China (Lin et al., 2021). Additionally, the resistance rates of 50 non-*agalactiae* streptococci isolates were 42% in penicillin, 40% in ampicillin, 38% in lincomycin, and 4% in rifaximin, and no non-*agalactiae* streptococci isolate was resistant to amoxi/clav, cefalexin, and ceftiofur; this result was similar to the report from France (Poutrel et al., 2018). Notably, non-*agalactiae* streptococci isolates isolated from SCM showed higher resistance rates to most antimicrobials than these isolated from CM with the exceptions of isolates tested against rifaximin (Table 2).

Recently, several researchers reported the isolation of *Acinetobacter* spp. from mastitic milk samples (Gurung et al., 2013; Song et al., 2020; Cao et al., 2021). However, the role of *Acinetobacter* spp. in the development of mastitis remains unclear, and *Acinetobacter* spp. are regarded as a commensal bacterium in bovine milk

Table 1. Distribution (%) of MIC for *Staphylococcus* spp. (n = 449) from mastitic milk samples on 15 large dairy farms across 12 provinces in China[1]

Pathogen	Antimicrobial	Distribution of MIC (μg/mL)											Resistance rate (%)			MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
		<0.25	0.25	0.5	1	2	4	8	16	32	64	>64	SCM	CM	In total		
Staphylococcus aureus (n = 97)	Penicillin	10	**29**	8	14	7	9	7	2	7	3	1	91.43	85.19	89.69	1	32
	Ampicillin	4	30	**11**	11	10	9	12	3	4	3	0	61.43	74.07	64.95	1	16
	Amoxi/clav	ND	53	24	14	5	0	**1**	0	0	0	0	0.00	3.70	1.03	<0.25	1
	Oxacillin	ND	54	31	9	0	**0**	0	1	0	0	2	0.00	11.11	3.09	<0.25	1
	Cefalexin	ND	59	17	17	1	0	1	1	**0**	0	1	0.00	3.70	1.03	<0.25	1
	Ceftiofur	ND	7	8	39	30	11	**0**	1	0	0	1	1.43	3.70	2.06	1	4
	Lincomycin	14	54	5	**0**	0	1	0	0	4	7	12	24.28	25.93	24.74	0.25	>64
	Rifaximin	38	47	10	**1**	0	1	0	0	0	0	0	0.00	7.41	2.06	0.25	0.5
CNS (n = 352)	Penicillin	61	**209**	37	20	13	6	1	1	2	2	0	97.42	53.78	82.67	0.25	1
	Ampicillin	37	189	**64**	32	16	8	1	1	2	1	1	35.20	36.97	35.80	0.25	1
	Amoxi/clav	ND	291	39	12	6	3	**1**	0	0	0	0	0.00	0.84	0.28	<0.25	0.5
	Oxacillin	ND	176	**85**	59	18	4	2	4	1	0	3	73.82	3.36	50.00	<0.25	1
	Cefalexin	ND	254	44	30	9	7	1	5	**1**	0	1	0.43	0.84	0.57	<0.25	1
	Ceftiofur	ND	99	69	100	51	23	**7**	1	0	1	1	2.14	4.20	2.84	1	2
	Lincomycin	72	173	31	**25**	15	5	3	5	3	4	16	21.03	22.69	21.59	0.25	4
	Rifaximin	145	154	32	**15**	3	0	0	0	2	0	1	6.87	4.20	5.97	0.25	0.5

[1]SCM = subclinical mastitis. CM = clinical mastitis. Amoxi/clav = amoxicillin-clavulanic acid (2:1). The MIC of amoxi/clav was represented as the concentration for amoxicillin. ND = not detected. Bold text represents the breakpoint for *Staphylococcus* spp. to a certain antimicrobial.

Table 2. Distribution (%) of MIC for *Streptococcus* spp. (n = 88) from mastitic milk samples on 15 large dairy farms across 12 provinces in China[1]

Pathogen	Antimicrobial	Distribution of MIC (μg/mL)											Resistance rate (%)			MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
		<0.25	0.25	0.5	1	2	4	8	16	32	64	>64	SCM	CM	In total		
Streptococcus agalactiae (n = 38)	Penicillin	3	32	**0**	1	1	1	0	0	0	0	0	3.84	16.67	7.89	0.25	0.25
	Ampicillin	0	24	**11**	1		1	1	0	0	0	0	30.77	50.00	36.84	0.25	0.5
	Amoxi/clav	ND	36	2	0	0	0	**0**	0	0	0	0	0.00	0.00	0.00	<0.25	<0.25
	Oxacillin	ND	5	30	1	1	1	0	0	0	0	0	—	—	—	0.5	0.5
	Cefalexin	ND	23	14	0	0	0	0	1	**0**	0	0	0.00	0.00	0.00	<0.25	0.5
	Ceftiofur	ND	34	2	2	0	0	**0**	0	0	0	0	0.00	0.00	0.00	<0.25	0.5
	Lincomycin	3	18	0	**0**	1	1	0	0	2	2	11	53.85	25.00	44.74	0.25	>64
	Rifaximin	12	25	1	**0**	0	0	0	0	0	0	0	0.00	0.00	0.00	0.25	0.25
Non-*agalactiae* streptococci (n = 50)	Penicillin	7	22	**11**	2	7	1	0	0	0	0	0	51.61	26.32	42.00	0.25	2
	Ampicillin	7	23	**8**	5	5	1	1	0	0	0	0	51.61	21.05	40.00	0.25	2
	Amoxi/clav	ND	39	4	5	1	1	**0**	0	0	0	0	0.00	0.00	0.00	<0.25	1
	Oxacillin	ND	15	3	4	16	6	1	5	0	0	0	—	—	—	2	16
	Cefalexin	ND	25	10	4	6	3	1	1	**0**	0	0	0.00	0.00	0.00	0.5	4
	Ceftiofur	ND	38	6	3	3	0	**0**	0	0	0	0	0.00	0.00	0.00	<0.25	1
	Lincomycin	11	19	1	**3**	9	2	0	0	1	1	3	45.16	26.32	38.00	0.25	32
	Rifaximin	16	29	3	**2**	0	0	0	0	0	0	0	0.00	10.53	4.00	0.25	0.5

[1]SCM = subclinical mastitis. CM = clinical mastitis. Amoxi/clav = amoxicillin-clavulanic acid (2:1). The MIC of amoxi/clav was represented as the concentration for amoxicillin. ND = not detected. — = no breakpoints. Bold text represents the breakpoint for *Streptococcus* spp. to a certain antimicrobial.

Table 3. Distribution (%) of MIC for *Acinetobacter* spp., *Escherichia* spp., and *Klebsiella* spp. (n = 136) from mastitic milk samples on 15 large dairy farms across 12 provinces in China[1]

Pathogen	Antimicrobial	Distribution of MIC (μg/mL)												Resistance rate (%)			MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
		<0.25	0.25	0.5	1	2	4	8	16	32	64	128	>128	SCM	CM	In total		
Acinetobacter spp.	Ampicillin	0	4	12	2	5	1	5	9	4	7	3	0	—	—	—	2	64
(n = 52)	Amoxi/clav	0	14	8	2	2	2	9	7	7	0	1	0	—	—	—	2	32
	Ceftiofur	0	0	2	7	7	7	5	8	10	3	2	1	—	—	—	8	64
	Rifaximin	0	11	6	5	7	18	2	0	0	2	1	0	—	—	—	2	4
Escherichia spp.	Ampicillin	0	1	1	1	5	11	8	1	**1**	3	1	10	40.74	28.57	34.88	8	>128
(n = 43)	Amoxi/clav	0	2	0	1	13	12	8	6	0	**1**	0	0	0.00	7.14	2.33	4	16
	Ceftiofur	0	14	21	1	2	1	**1**	1	1	0	1	0	11.11	7.14	9.30	0.5	4
	Rifaximin	1	1	0	0	1	5	10	11	11	0	2	1	—	—	—	16	32
Klebsiella spp.	Amoxi/clav	0	1	3	12	11	5	2	0	**1**	5	1	0	17.39	15.00	17.07	2	64
(n = 41)	Ceftiofur	1	15	12	5	1	1	0	3	1	0	2	0	—	—	—	0.5	16
	Rifaximin	0	1	0	1	2	4	6	9	10	1	3	4	—	—	—	16	128

[1]SCM = subclinical mastitis. CM = clinical mastitis. Amoxi/clav = amoxicillin-clavulanic acid (2:1). The MIC of amoxi/clav was represented as the concentration for amoxicillin. — = no breakpoints. Bold text represents the breakpoint for *Acinetobacter* spp. or *Escherichia* spp. or *Klebsiella* spp. to a certain antimicrobial.

(Catozzi et al., 2017). We determined the MIC for 52 *Acinetobacter* spp. isolates (Table 3). In the absence of breakpoints for *Acinetobacter* spp., only MIC_{50} and MIC_{90} were reported. We found that both the MIC_{50} and MIC_{90} of *Acinetobacter* spp. against all the tested antimicrobials greatly varied.

The most common gram-negative mastitis pathogens are *E. coli* and *Klebsiella* spp. (Schukken et al., 2012; Klaas and Zadoks, 2018). A total of 43 *Escherichia* spp. isolates were tested for antimicrobial susceptibility (Table 3). The resistance rates for *Escherichia* spp. were as follows: ampicillin (34.88%), ceftiofur (9.30%), and amoxi/clav (2.33%). Notably, *Escherichia* spp. isolates isolated from SCM showed higher resistance rates to most antimicrobials than these isolated from CM with exceptions of isolates tested against amoxi/clav (Table 3).

Similarly, 30.1% of *E. coli* isolates were resistant to ampicillin (Yu et al., 2020). However, only 11.5% of *E. coli* isolates from mastitic dairy cows in Canada were resistant to ampicillin (Majumder et al., 2021). Moreover, other reports in China showed that 81% and 16% of *E. coli* isolates from CM were resistant to amoxi/clav and ceftiofur, respectively (Cheng et al., 2019).

Compared with *E. coli*, the lack of effective treatments makes *Klebsiella* spp. mastitis especially troublesome. Consequently, the duration of milk production loss was longer in cases of *Klebsiella* spp. (Schukken et al., 2012; Klaas and Zadoks, 2018). In this study, a total of 41 *Klebsiella* spp. isolates were tested for antimicrobial susceptibility (Table 3). Approximately 17.07% of *Klebsiella* spp. isolates were resistant to amoxi/clav. However, a higher resistance (38%) to amoxi/clav was reported in China (Cheng et al., 2019).

The variations in resistance rates of the most frequently isolated mastitis pathogens among different reports potentially stemmed from a variety of factors, such as the enforcement of national guidelines for appropriate antimicrobial use, medication practices, geographic location, seasonal factors, milk samples obtained from cows with different severities of mastitis, and specific antimicrobial requirements in different countries.

In conclusion, our study showed that CNS (52.30%, 352/673) was the predominant pathogen, followed by *Staph. aureus* (14.41%, 97/673), *Acinetobacter* spp. (7.72%, 52/673), and non-*agalactiae* streptococci (7.42%, 50/673). The *Staph. aureus*, CNS, and non-*agalactiae* streptococci exhibited the highest resistance rates to penicillin (89.69%, 82.67%, and 42.00%, respectively); on large Chinese dairy farms, the resistance of *Streptococcus* spp. against lincomycin was the most prevalent (44.74%). Notably, most mastitis pathogens isolated from CM showed higher resistance rates to most antimicrobials than these isolated from SCM. Overall, compared with other reports, the resistance rates of the bovine mastitis pathogens isolated in our study were relatively moderate. Consequently, implementing a nationwide surveillance program for AMR of bovine mastitis pathogens is imperative to improve treatment effectiveness and control the development of AMR in Chinese dairy farms.

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Notes

Xiangbin Song https://orcid.org/0000-0001-8068-6089
Yaxin Wang https://orcid.org/0009-0001-9869-4420
Rina Bai https://orcid.org/0009-0009-4939-9888
Hongyan Xu https://orcid.org/0009-0005-4404-822X
Congming Wu https://orcid.org/0000-0002-4238-9080

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