Bacteria from Bovine Clinical Mastitis Showed Multiple Drug Resistance

Sudhakar P Awandkar (✉ drspawandkar@gmail.com)

College of Veterinary and Animal Sciences Udgir <https://orcid.org/0000-0002-7171-8576>

Mahesh B Kulkarni

College of Veterinary and Animal Sciences Udgir

Research Article

Keywords: MDR, Clinical, Mastitis, Bovine, India

Posted Date: May 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-356653/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Veterinary Research Communications on September 27th, 2021. See the published version at <https://doi.org/10.1007/s11259-021-09838-8>.

Abstract

Mastitis, most often udder infection of dairy animals attracted concerns due to heavy economic loss to dairy industry and public health. This study was conducted to determine the cultivable bacterial species associated with bovine clinical mastitis and their resistance patterns to different antimicrobials. The milk samples from 272 quarters of cows suffering from clinical mastitis were investigated. A total of 110 bacterial isolates belonging to 14 different genera were isolated and identified. Aminoglycosides and Quinolones were found to be most effective antibiotics. We demonstrated Extended Spectrum β Lactamases (ESBL), Cephalosporins, Tetracyclines, Vancomycin and Chloramphenicol resistant Gram-positive and Gram-negative bacteria along with Vancomycin Resistant Enterococci (VRE), Multiple Drug Resistant Gram-Negative Rods (MDR-GNR), MDR-Pseudomonas (MDR-P) and MDR Acinetobacter (MDR-A). The ESBLs and cephalosporins resistant *S. aureus* isolates showed resistance to Vancomycin. Wide spread of resistance among *Streptococcus uberis* against ESBLs and Cephalosporins, widely used antibiotics in bovine mastitis, was documented. Variable MDR patterns were recorded for every species. MDR transfer from non-pathogens to emerging foodborne and established mastitis pathogens could be potential problem to dairy industry as well as public health.

Introduction

Bovine mastitis is professed to be the leading affection influencing productivity and health of dairy animals (Hogeveen et al. 2011). It is a complex multi-factorial illness caused by variety of pathogens including bacteria, viruses and fungi. Its occurrence may vary depending upon the animals, pathogens involved and environment (Constable et al. 2017). Variety of bacterial agents associated with bovine mastitis are commonly isolated from dairy animals' environment (Bradley 2012).

Streptococcus, *Staphylococcus* and *Corynebacterium*, spp. are potential bacteria causing clinical as well as subclinical mastitis. In addition, various environmentally originated aetiology including *Escherichia*, *Acinetobacter*, *Pasteurella*, *Pseudomonas*, *Klebsiella*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Enterococcus* and *Serratia* are involved in aggravated conditions. These are often seen to carry antimicrobial resistance (AMR). These pathogens are developing multiple drug resistance (MDR) to popular antimicrobial agents used in mastitis therapy (Weisblum 1995; McMurry and Levy 2000; Botrel 2010). Irrational use of antibiotics leads to mutations in bacteria allowing them to survive, making treatment more complicated and costly affair. However, bacterial isolates associated with bovine mastitis were dramatically found susceptible to Gentamicin (Awandkar et al. 2009; Salih and Gibreel 2019). The sporadic literature is available illustrating extensive studies on MDR in Indian dairy animals. Therefore, the study was planned to decipher the antimicrobial resistance profile of bacterial pathogens associated with clinical bovine mastitis in dairy cows.

Materials And Methods

Milk Sampling

The milk samples from 272 quarters of lactating cows exhibiting clinical mastitis were collected from 7 districts of western India during 2018 to 2020. First three to four strips were discarded and next two stripping were collected in sterile container. The samples were brought to the Microbiology Laboratory in ice-cooled containers. The samples were processed within 24 hrs of collection.

Isolation of bacteria

Each of the sample was inoculated by streaking on 5% sheep blood agar and incubated aerobically for 24–48 hrs at 37°C. The colonies of suspicious bacterial pathogens were further sub cultured on Brain Heart Infusion Agar for lysate preparation and antimicrobial susceptibility testing.

Identification of bacteria

The bacterial isolates were identified by observing nature of growth, cultural characteristics, Gram's staining followed by MALDI-TOF MS analysis (Bruker Daltonics, Bremen, Germany).

The protein was extracted from bacterial cultures using formic acid and analysed by MALDI-TOF MS as per the procedure recommended by Bruker Daltonics. The bacterial growth was pelleted by centrifugation. The bacterial pellet was mixed with 1 ml of 70% ethanol and centrifuged at 13,000 g for 2 min. The supernatant was discarded. The pellet was dissolved in 25 µl of 70% formic acid and 25 µl of acetonitrile. The mixture was centrifuged at 13,000 g for 2 min. The supernatant (1 µl) containing the bacterial extract was transferred onto the MALDI target plate and allowed to dry at room temperature. After drying the sample spot was overlaid with 1 µl of MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and dried in the air. Mass spectra were then acquired by Microflex MALDI-TOF MS. MALDI BioTyper 2.0 software was used for spectral analysis and comparison with the MALDI BioTyper database. The score ≥ 2 was considered as valid for species identification while the score between 1.7 and 2 was considered as valid for genus identification. Score ≤ 1.7 was considered invalid.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test of all the bacterial isolates was performed by the Kirby Bauer agar disc diffusion method (Clinical Laboratory Standards Institute 2019) using Mueller Hinton agar (HiMedia, India). Commercially available 18 antibiotic discs (Hi-Media, India) viz. Amikacin (30 mcg), Amoxicillin-sulbactam (30/15 mcg), Azithromycin (15 .mcg), Cefotaxime (30 mcg), Ceftriaxone-tazobactam (30/10 mcg), Ciprofloxacin (5 mcg), Chloramphenicol (30 mcg), Doxycycline (30 mcg), Enrofloxacin (10 mcg), Erythromycin (15 mcg), Furazolidone (100 mcg), Gentamicin (10 mcg), Lincomycin (15 mcg), Nitrofurantoin (300 mcg), Oxytetracycline (30 mcg), Penicillin (10 units), Sulfadiazine (100 mcg) and Vancomycin (30 mcg) belonging to 11 antibiotic groups were involved in the study. *Escherichia coli* (ATCC 4388), *Pasteurella multocida* subsp. *multocida* (ATCC 12945), *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 11774) were used as control strains.

The bacterial isolates were inoculated in Brain heart infusion broth and incubated at 37°C for 4 to 10 hours until turbidity developed to 0.5 McFarland's standard. The inoculum was spread onto the entire surface of sterile MHA medium with sterile cotton swabs. The plate was incubated at room temperature for 10 minutes before applying antibiotic discs. The plates were incubated at 37°C for 16 hours. The average of at least two perpendicular diameters of growth inhibition zones was recorded.

Results

The milk samples from 272 quarters of cows suffering from clinical mastitis were investigated. A total of 110 bacterial isolates belonging to 14 different genera were isolated (Table 1 and Fig. 1). The antimicrobial resistance of the bacterial isolates to different antibiotics is compared in Fig. 2, Table 2 and 3.

Table 1
Details of the bacterial isolates obtained from bovine clinical mastitis

SN	Genera	Bacterial isolates	MALDI-TOF Score	Total No.	%
1	Acinetobacter	Acinetobacter baumannii	2.213	5	5.50
		Acinetobacter pittii	2.115	1	
2	Arthrobacter	Arthrobacter arilaitensis	2.421	1	0.92
3	Bacillus	Bacillus cereus	2.402	5	7.34
		Bacillus spp.	1.842	2	
		Bacillus subtilis	2.087	1	
4	Corynebacterium	Corynebacterium casei	2.588	1	1.83
		Corynebacterium spp.	1.986	1	
5	Enterobacter	Enterobacter asburiae	2.379	1	4.59
		Enterobacter cancerogenus	2.314	1	
		Enterobacter cloacae	2.243	3	
6	Enterococcus	Enterococcus casseliflavus	2.245	2	10.09
		Enterococcus faecalis	2.440	3	
		Enterococcus faecium	2.308	1	
		Enterococcus gallinarum	2.213	5	
7	Escherichia	Escherichia coli	2.475	9	9.17
		Escherichia hermannii	2.441	1	
8	Klebsiella	Klebsiella pneumoniae	2.483	3	2.75
9	Lactococcus	Lactococcus lactis	2.475	2	1.83
10	Pasteurella	Pasteurella spp.	1.969	1	1.83
		Pasteurella multocida	2.443	1	
11	Pseudomonas	Pseudomonas aeruginosa	2.445	8	10.09
		Pseudomonas mosselii	2.440	1	
		Pseudomonas putida	2.208	2	
12	Serratia	Serratia marcescens	2.253	2	1.83
13	Staphylococcus	Staph. aureus	2.485	10	35.78
		Staph. arlettae	2.341	10	

SN	Genera	Bacterial isolates	MALDI-TOF Score	Total No.	%
		Staph. chromogenes	2.488	2	
		Staph. cohnii sp. urealyticus	2.294	2	
		Staph. epidermidis	2.341	2	
		Staph. haemolyticus	2.445	3	
		Staph. hominis	2.475	1	
		Staph. pseudoepidemidis	2.314	1	
		Staph. saprophyticus	2.308	2	
		Staph. sciuri	2.214	4	
		Staph. simulans	2.336	2	
14	Streptococcus	Strep. uberis	2.389	6	7.34
		Strep. spp.	1.795	2	

Table 2

MDR patterns shown by Gram-positive bacterial isolates obtained from bovine clinical mastitis

SN	Isolates	n	MDR Patterns
1	Staph. aureus	1	LIN, OTC, DOX, AMK, GEN, ENR, CIP, NFT, FUR, CFO, CFT, AZI, ERY
		2	VAN, LIN, OTC, ENR, FUR, CFO, CFT, ERY, PEN, AMS
		3	VAN, LIN, OTC, FUR, CFO, CFT, ERY, PEN, AMS
		2	CHL, VAN, NFT, CFO, CFT, ERY, PEN
		4	VAN, AMK, CFO, CFT, ERY, PEN
		4	OTC, FUR, CFO, CFT, ERY, PEN
		3	VAN, ENR, CFO, CFT, ERY, PEN
		3	VAN, NFT, CFO, CFT, ERY, PEN
	Staph. arlettae	1	LIN, OTC, DOX, FUR, CFO, CFT, AZI, PEN
		1	OTC, FUR, CFO, CFT, AZI, PEN, AMS
		1	CHL, SDZ, CFO, AZI, ERY, PEN, AMS
		1	CHL, OTC, FUR, CFO, AZI, ERY, PEN
		4	OTC, FUR, AZI, PEN
		5	CFO, CFT, AZI, PEN
	Staph. sciuri	2	CHL, LIN, SDZ, OTC, DOX, FUR, CFO, CFT, AZI, ERY, PEN
2	Strep. uberis	1	CHL, VAN, LIN, SDZ, OTC, CIP, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		2	CHL, VAN, LIN, OTC, NFT, FUR, CFO, ERY, PEN, AMS
		2	OTC, AMK, ENR, CIP, FUR, CFO, CFT, PEN
		3	VAN, LIN, OTC, FUR, CFO, ERY, PEN, AMS
		3	OTC, DOX, ENR, CIP, FUR, CFO, PEN
		3	OTC, ENR, CIP, FUR, CFO, CFT, PEN
		1	FUR, CFO, CFT, AZI, ERY, PEN, AMS
		4	OTC, ENR, CIP, FUR, CFO, PEN
3	Corynebacterium spp.	1	CHL, SDZ, OTC, FUR, CFO, ERY, PEN
4	Enterococcus spp.	1	VAN, LIN, SDZ, OTC, DOX, AMK, ENR, NFT, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		1	VAN, LIN, SDZ, OTC, DOX, AMK, FUR, CFO, CFT, AZI, ERY, PEN, AMS

SN	Isolates	n	MDR Patterns
		1	VAN, LIN, SDZ, AMK, GEN, ENR, NFT, CFO, CFT, ERY, PEN
		2	CHL, VAN, LIN, SDZ, NFT, CFO, CFT, ERY, PEN, AMS
		2	LIN, SDZ, OTC, DOX, AMK, CFO, CFT, AZI, ERY, PEN, AMS
		3	LIN, SDZ, OTC, DOX, AMK, FUR, CFO, CFT, AZI, ERY, PEN
		5	VAN, LIN, SDZ, CFO, CFT, ERY, PEN
		3	SDZ, AMK, ENR, CFO, CFT, ERY, PEN
		8	SDZ, CFO, CFT, ERY, PEN
		5	ENR, CFO, CFT, ERY, PEN
5	Bacillus cereus	1	CHL, VAN, LIN, SDZ, OTC, DOX, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		1	CHL, VAN, LIN, OTC, DOX, NFT, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		3	CHL, VAN, LIN, OTC, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		2	SDZ, OTC, DOX, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		5	OTC, NFT, FUR, CFO, CFT, PEN, AMS
		4	LIN, NFT, CFO, ERY, PEN, AMS
		3	SDZ, NFT, CFO, CFT, PEN, AMS
6	Lactococcus lactis	1	CHL, LIN, OTC, DOX, FUR, CFO, CFT, AZI, ERY, PEN
		1	VAN, SDZ, OTC, DOX, FUR, CFO, PEN
		1	VAN, SDZ, AMK, NFT, PEN

Table 3
MDR patterns of Gram-negative bacterial isolates obtained from bovine clinical mastitis

SN	Isolates	n	MDR Patterns
1	Escherichia coli	2	CHL, VAN, LIN, SDZ, OTC, DOX, ENR, CIP, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		3	VAN, LIN, SDZ, OTC, DOX, ENR, CIP, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		4	VAN, LIN, SDZ, OTC, DOX, ENR, CIP, FUR, CFO, CFT, ERY, PEN, AMS
		1	VAN, LIN, OTC, AMK, FUR, CFO, CFT, ERY, PEN, AMS
		3	VAN, LIN, NFT, CFO, CFT, ERY, PEN, AMS
		5	VAN, LIN, AMK, CFO, CFT, PEN, AMS
		5	VAN, LIN, OTC, CFO, CFT, ERY, PEN
		7	VAN, LIN, CFO, CFT, PEN, AMS
		6	VAN, LIN, OTC, FUR, ERY, PEN
	Escherichia hermannii	1	CHL, VAN, LIN, SDZ, OTC, DOX, AMK, NFT, FUR, CFO, CFT, AZI, PEN
2	Pseudomonas aeruginosa	3	CHL, VAN, LIN, SDZ, OTC, DOX, NFT, FUR, CFO, ERY, PEN, AMS
		3	CHL, VAN, LIN, SDZ, OTC, DOX, CFT, FUR, CFO, ERY, PEN, AMS
		6	CHL, VAN, LIN, SDZ, OTC, DOX, FUR, CFO, ERY, PEN, AMS
		7	CHL, VAN, LIN, SDZ, OTC, DOX, FUR, PEN, AMS
	Other Pseudomonas	1	CHL, VAN, LIN, SDZ, OTC, DOX, NFT, FUR, CFO, CFT, AZI, ERY, PEN, AMS
3	Klebsiella pneumoniae	1	VAN, LIN, OTC, AMK, CIP, NFT, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		1	VAN, LIN, SDZ, AMK, CIP, NFT, CFO, AZI, ERY, PEN, AMS
		2	VAN, LIN, AMK, CIP, NFT, CFO, ERY, PEN, AMS
		2	VAN, LIN, AMK, CIP, CFO, CFT, ERY, PEN, AMS
		2	VAN, LIN, AMK, CIP, CFO, AZI, ERY, PEN, AMS
		3	VAN, LIN, AMK, CIP, CFO, ERY, PEN, AMS
4	Pasteurella spp.	2	VAN, LIN, OTC, DOX, AMK, GEN, CIP, FUR, CFO, CFT, ERY, PEN, AMS
5	Serratia marcescens	1	CHL, VAN, LIN, SDZ, OTC, DOX, AMK, NFT, FUR, CFO, CFT, ERY, PEN, AMS

SN	Isolates	n	MDR Patterns
		2	VAN, LIN, OTC, DOX, AMK, NFT, FUR, CFO, CFT, ERY, PEN, AMS
6	Acinetobacter baumannii	1	CHL, VAN, LIN, SDZ, OTC, GEN, ENR, CIP, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		1	VAN, LIN, SDZ, OTC, DOX, AMK, NFT, FUR, CFO, CFT, AZI, ERY, PEN, AMS
		1	VAN, LIN, SDZ, OTC, AMK, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		1	CHL, VAN, LIN, OTC, AMK, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		4	CHL, VAN, LIN, OTC, NFT, CFO, CFT, ERY, PEN, AMS
7	Enterobacter spp.	2	VAN, LIN, OTC, DOX, AMK, NFT, FUR, CFO, CFT, ERY, PEN, AMS
		4	VAN, LIN, AMK, NFT, PEN, AMS
		4	VAN, LIN, OTC, NFT, PEN, AMS
		4	VAN, LIN, DOX, NFT, PEN, AMS
		5	VAN, LIN, NFT, PEN, AMS

Among these isolates, *Staphylococcus aureus* and non-*Staphylococcus aureus* (NSA) were emerged as principal pathogen associated with bovine mastitis (35.78%) followed by *Pseudomonas* spp. and *Enterococcus* spp. (10.09% each), *Escherichia* spp. (9.17%), *Streptococcus* spp. and *Bacillus* spp. (7.34% each), *Acinetobacter* spp. (5.50%), *Enterobacter* spp. (4.59%), *Klebsiella* spp. (2.75%) *Serratia* spp., *Lactococcus* spp., *Corynebacterium* spp. and *Pasteurella* spp. (1.83% each) and *Arthrobacter* spp. (0.92%).

Staphylococcus spp.

Staphylococcus spp. showed resistance to all the antibiotics evaluated in this study. They developed almost resistance to penicillin (97.44%) and more than 50% isolates exhibited resistance to most popularly used antibiotics in the field viz. cefotaxime and ceftriaxone-tazobactam. The marked resistance was recorded to other antibiotics which are less frequently used for treatment of clinical mastitis viz. oxytetracycline (41.03%), amoxicillin-sulbactam (33.33%), enrofloxacin, amikacin, chloramphenicol (15.38%) and doxycycline (12.82%). We could demonstrate resistance of these bacteria to other antibiotics like erythromycin (64.10%), furazolidone (43.59%), azithromycin (38.46%), vancomycin (33.33%), lincomycin (20.51%), nitrofurantoin (117.95%) and sulfadiazine (12.82%) which are never used for the treatment of mastitis in the study area. Only gentamicin (97.44%) and ciprofloxacin (94.87%) were found most effective antibiotics against *Staphylococcus* spp. with least resistance.

Most of the *S. aureus* isolates were found to be MDR. Diverged MDR patterns were recorded. All of the isolates showed resistance to β Lactamase and ESBL group of antibiotics. These isolates were also

found to be resistant to vancomycin. Lincomycin-Tetracycline-Aminoglycosides-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides resistance pattern was found in one isolate. Glycopeptides-Lincomycin-Tetracycline-Quinolones-Nitrofurantoin- Cephalosporin-Macrolides- β Lactamases and Chloramphenicol-Glycopeptides-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases resistance patterns were recorded in two isolates each. Three isolates showed resistance to Glycopeptides-Lincomycin-Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases, Glycopeptides-Quinolones-Cephalosporin-Macrolides- β Lactamases and Glycopeptides-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases groups of antibiotics while four isolates showed Glycopeptides- Aminoglycosides–Cephalosporin-Macrolides- β Lactamases and Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases resistance patterns.

Enterococcus spp.

Enterococcus spp. also showed resistance to all the antibiotics under study. Penicillin, ceftriaxone-tazobactam and cefotaxime were found to be totally ineffective against Enterobacter isolates recovered from milk samples. Most of the isolates developed resistance to erythromycin and vancomycin (81.82%) followed by lincomycin and sulfadiazine (72.73%), amoxicillin-sulbactam and amikacin (63.64%), azithromycin and nitrofurantoin (54.55%), enrofloxacin and oxytetracycline (45.45%), furazolidone and doxycycline (36.36%), chloramphenicol and ciprofloxacin (18.18%) and gentamicin (9.09%).

At least one Enterococcus isolates showed MDR to Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Aminoglycosides-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases, Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Aminoglycosides-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases and Glycopeptides-Lincomycin-Sulfonamide-Aminoglycosides-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases. Chloramphenicol-Glycopeptides-Lincomycin-Sulfonamide-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases and Lincomycin-Sulfonamide-Tetracycline-Aminoglycosides-Cephalosporin-Macrolides- β Lactamases resistance pattern was recorded for two isolates each. Similarly, three isolates showed Lincomycin-Sulfonamide-Tetracycline-Aminoglycosides-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases resistance pattern.

Streptococcus spp.

Streptococcus spp. showed resistance to all the antibiotics except gentamicin. All the isolates were sensitive to gentamicin and completely resistant to penicillin, cefotaxime and furazolidone. The isolates were resistant to commonly used antibiotics in livestock therapy viz. oxytetracycline (85.71%), ceftriaxone-tazobactam and ciprofloxacin (71.43%), amoxicillin-sulbactam, enrofloxacin and erythromycin (57.14%), doxycycline, vancomycin and lincomycin (42.86%), nitrofurantoin, amikacin and chloramphenicol (28.57%), azithromycin and sulfadiazine (14.29%).

MDR patterns observed for Chloramphenicol-Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases and Nitrofurantoin- Cephalosporin-

Macrolides-β Lactamases were restricted to one isolate of *Streptococcus uberis* each. However, two isolates showed Chloramphenicol-Glycopeptides-Lincomycin-Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides-β Lactamases and Tetracycline-Aminoglycoside-Quinolones-Nitrofurantoin-Cephalosporin-β Lactamases resistance patterns. Glycopeptides-Lincomycin-Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides-β Lactamases, Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-β Lactamases and Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-β Lactamases resistance patterns were exhibited by three isolates each. Four MDR isolates indicated Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-β Lactamases resistance pattern.

Bacillus spp.

The *Bacillus* isolates did not show resistance to amikacin, gentamicin, enrofloxacin and ciprofloxacin while least resistance was recorded to azithromycin (12.50%), doxycycline (25%), chloramphenicol and sulfadiazine (37.50%). Most of the isolates were resistant to oxytetracycline, vancomycin and lincomycin (62.50%), erythromycin, furazolidone and ceftriaxone-tazobactam (75%), amoxicillin-sulbactam and nitrofurantoin (87.50%). The bacteria indicated complete resistance to cefotaxime and penicillin.

Bacillus cereus isolates (one each) showed MDR to 13 antibiotics distributed over nine and eight antibiotic groups. Most of the isolates showed Cephalosporins-β Lactamases-Macrolides-Nitrofurantoin resistance pattern.

Pseudomonas spp.

Pseudomonas isolates were found to be completely resistant to furazolidone and lincomycin. More than 90% isolates showed resistance to chloramphenicol, vancomycin, sulfadiazine, oxytetracycline, doxycycline, penicillin and amoxicillin-sulbactam, 81.82% to cefotaxime and erythromycin, 63.64% to nitrofurantoin, 54.55% to ceftriaxone-tazobactam, 36.36% to azithromycin and 27.27% to amikacin. All the isolates were found to be sensitive to gentamicin and enrofloxacin and 90.01% to ciprofloxacin.

Pseudomonas aeruginosa were recorded high MDR with Chloramphenicol-Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides-β Lactamases resistance pattern. These MDR *Pseudomonas* were found to be sensitive to Aminoglycosides and Quinolones.

Escherichia spp.

More than 30% *Escherichia* isolates showed resistance to all the antibiotics except gentamicin. All the isolates exhibited resistance to penicillin, lincomycin and vancomycin followed by cefotaxime (90%), ceftriaxone-tazobactam, oxytetracycline and erythromycin (80%), amoxicillin-sulbactam and furazolidone (70%), amikacin (60%), azithromycin, doxycycline and sulfadiazine (50%), enrofloxacin, ciprofloxacin and nitrofurantoin (40%) and chloramphenicol (30%).

MDR patterns recorded for *E. coli* isolates were Chloramphenicol-Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (2 isolates), Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (4 isolates), Glycopeptides-Lincomycin-Tetracycline-Aminoglycosides-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (1 isolate), Glycopeptides-Lincomycin-Aminoglycosides-Cephalosporin-Macrolides- β Lactamases (5 isolates). Other isolates showed MDR to at least four groups of antibiotics.

Acinetobacter spp.

Acinetobacter spp. showed resistance to all the antibiotics with complete resistance to nine antibiotics distributed over seven groups (oxytetracycline, vancomycin, lincomycin, nitrofurantoin, ceftriaxone-tazobactam, cefotaxime, erythromycin, amoxicillin-sulbactam and penicillin). Least resistance was exhibited to doxycycline, gentamicin, enrofloxacin, azithromycin and ciprofloxacin (16.67%) while 50%, 66.67% and 83.33% isolates were found to be resistant to amikacin, chloramphenicol and sulfadiazine and furazolidone, respectively.

Acinetobacter baumannii, udder inhabitant, showed considerable MDR with variable patterns. Chloramphenicol-Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Aminoglycosides-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (2 isolates), Glycopeptides-Lincomycin-Sulfonamide-Tetracycline-Quinolones-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (2 isolates) and Chloramphenicol-Glycopeptides-Lincomycin-Tetracycline-Nitrofurantoin-Cephalosporin-Macrolides- β Lactamases (4 isolates) MDR patterns were recorded.

Enterobacter spp.

Penicillin, amoxicillin-sulbactam, vancomycin, lincomycin and nitrofurantoin were totally ineffective against *Enterobacter* isolates recovered from milk samples. Most of the *Enterobacter* isolates developed resistance against oxytetracycline, doxycycline and amikacin (80%), ceftriaxone-tazobactam and cefotaxime (60%) while 40% isolates were resistant to erythromycin and furazolidone and 20% to azithromycin and gentamicin. Chloramphenicol, sulfadiazine, enrofloxacin and ciprofloxacin were completely effective against the *Enterobacter* isolates.

Enterobacter isolates showed common MDR to antimicrobials from Glycopeptides-Lincomycin-Tetracycline-Nitrofurantoin- β Lactamases groups. However, an isolate showed additional resistance to antibiotics belonging to the groups Aminoglycosides and Macrolides. Another isolate was found to be sensitive to Tetracycline group of antibiotics.

Other bacteria

Klebsiella isolates showed complete resistance to vancomycin, lincomycin, amikacin, ciprofloxacin, cefotaxime, erythromycin, penicillin and amoxicillin-sulbactam. They were found sensitive to chloramphenicol and enrofloxacin only. MDR *Klebsiella pneumoniae* showed resistance to Glycopeptides-Lincomycin-Aminoglycosides-Quinolones-Cephalosporin-Macrolides- β Lactamases groups (3 isolates) with additional resistance to tetracycline (1 isolate) and Sulphonamide (1 isolate).

Pasteurella isolates exhibited total resistance to vancomycin, lincomycin, oxytetracycline, doxycycline, amikacin, enrofloxacin, ciprofloxacin, furazolidone, cefotaxime, ceftriaxone-tazobactam, erythromycin, penicillin and amoxicillin-sulbactam and sensitivity to chloramphenicol, azithromycin, gentamicin, sulfadiazine and nitrofurantoin.

Corynebacterium isolates showed sensitivity to amoxicillin-sulbactam, azithromycin, ceftriaxone-tazobactam, amikacin, gentamicin, doxycycline, enrofloxacin, vancomycin and lincomycin, while *Lactococcus* isolates showed complete resistance to penicillin only.

Serratia spp. were resistant to vancomycin, lincomycin, oxytetracycline, doxycycline, amikacin, nitrofurantoin, furazolidone, cefotaxime, ceftriaxone-tazobactam, erythromycin, penicillin and amoxicillin-sulbactam and sensitivity to azithromycin, gentamicin, enrofloxacin and ciprofloxacin.

Discussion

In clinical mastitis similar distribution of *Staphylococcus* spp., *Pseudomonas* spp. *Streptococcus* spp., *Pasteurella* spp., *Enterobacter* spp. *Klebsiella* spp. and *Corynebacterium* spp. were reported earlier (Das et al. 2017). In contrast, we report higher distribution of *Pseudomonas* spp. and *Enterococcus* spp. and comparatively less but variable distribution of *Staphylococcus* spp., *Escherichia* spp. and *Klebsiella* spp. as compare to earlier reports (Gundogan and Avci 2014; Jahan et al. 2015; Gao et al. 2017; Salauddin et al. 2020). Except *Staphylococcus* spp., other isolates including *Pseudomonas* spp., *Enterococcus* spp., *Escherichia* spp., *Streptococcus* spp. and *Bacillus* spp. were emerged as major bacteria associated with bovine mastitis in the study area. We could also isolate *Pseudomonas* spp. and *Acinetobacter* spp. having public health significance. Various workers reported less isolation rate of *Acinetobacter* spp. from milk samples which is in accordance with the findings of present study (Gurung et al 1993; Nam et al. 2010). Variation in distribution patterns of mastitis bacteria may indicate their geographical distribution, health status and biosecurity practices of the study area (Salaudin et al. 2019).

Similar findings were documented previously from Bangladesh (Jahan et al 2015; Salauddin et al. 2020) for *Staphylococci* spp. Comparable results were documented for *Bacillus* spp. earlier from Karnataka state indicating 91.40% resistance to penicillin followed by ampicillin (50.67%), Ceftriaxone (35.29%), Erythromycin (20.36%) and Azithromycin (5.42%), but all *Bacillus* spp. susceptible to Vancomycin contrary to present findings (Sadashiv and Kaliwal 2014). All the *Escherichia* isolates were sensitive to gentamicin. On the contrary, earlier reports suggested majority of *E. coli* isolates, from bovine mastitis, resistant to gentamicin and susceptible to gentamicin, amoxicillin and ciprofloxacin (Yang et al. 2018;

Lan et al. 2020). Low preference to gentamicin in animal therapy may be the reason behind high sensitivity.

Complete resistance to β Lactamases and MDR exhibited by *Staphylococci* spp. associated with bovine mastitis may be due to indiscriminate use of these antibiotics (Pitaka et al. 2004; Hendriksen et al. 2008; Kalmus et al. 2011) in therapy. The MDR staphylococci reported in this study showed intermediate resistance to vancomycin as reported earlier (Kateete et al. 2013; Nobrega et al. 2018). Use of vancomycin therapy for infections of gram-positive cocci is critical as they developed complete resistance to β Lactamases. However, increasing resistance to vancomycin may have implications on animal and human health.

Earlier report divergently suggested common resistance of *Streptococcus uberis* to tetracyclines followed by gentamicin and absence of resistance to penicillin and ampicillin (Minst et al. 2012). Our study reports relatively high MDR Streptococci with complete resistance to penicillin (Schukken et al. 2012) and intermediate resistance to vancomycin (Kateete et al. 2013; Nobrega et al. 2018). The report suggested that the β Lactamase resistant streptococci have acquired considerable resistance against vancomycin, and may have health worries in near future.

Bacillus cereus is considered as a common contaminant of milk as it is ubiquitously present in cow's environment and is not considered as primary pathogen causing mastitis (Sadashiv and Kaliwal 2014). It's abundant presence in soil and teats and accidental introduction in to the udder via teat canal may explain high isolation rate in present study. The results are indicative of lack of hygienic practices in dairy farming operations in the study area.

Earlier reports also suggested complete resistance exhibited by *Pseudomonas aeruginosa* against many antibiotics as documented in our study (Bernal-Rosas et al. 2015). β -Lactam antibiotics and cephalosporins were suggested to be essential components for treatment of *Pseudomonas aeruginosa* infection (Mesaros et al. 2007), however, development of high resistance to these antibiotic groups reported in the present report is alarming situation (Ranjan et al. 2010; Akhoun et al. 2012; Kotwal et al. 2016; Awad et al. 2017). Ciprofloxacin still remains effective (Hirakawa et al. 2010; Biswal et al. 2014) along with gentamicin and enrofloxacin.

Quinolones are widely used in veterinary and medical health care. However, high quinolone resistance in *E. coli* and other Gram-negative bacteria was documented earlier (Machuca et al. 2017) as reported in present study. Similarly, few reports suggesting ESBL producing and tetracycline resistant *E. coli* in bovine mastitis (Ghatak et al. 2013; Bandyopadhyay et al. 2015) support the present findings. Irrational antibiotic use may have contributed to increased resistance of MDR *E. coli* (Chandrasekaran et al. 2014; Hinthong et al. 2017). Moreover, the presence of antimicrobials in the environment of dairy animals may be the reason for MDR in *E. coli* which are ubiquitously distributed in cow's environment. The ability of producing β lactamase and penicillin binding proteins might have caused resistance to penicillin group of antibiotics (Kumar et al. 2010). Symptomatic therapy may be helpful in reducing clinical symptoms instead of antimicrobial treatment against MDR *E. coli* (Schukken et al. 2012).

The results indicated complete resistance of *Klebsiella* spp. to third generation cephalosporins, supporting indication from earlier workers that they will be ineffective against stabilized population *Klebsiella* spp. in most parts of the world by 2030 (Alvarez-Uria et al. 2018). Our results had higher MDR in *Klebsiella* spp. as reported earlier (Schukken et al. 2012; Alvarez-Uria et al. 2018) [27, 42]. In view of complete resistance to multiple antibiotic groups, therapy should be focussed to reduce clinical symptoms and to save cows suffering from clinical mastitis caused by MDR *Klebsiella* spp. (Schukken et al. 2012).

Use of antibiotics was suggested to be positively associated with antibiotic resistance in mastitis bacteria [43] therefore, their irrational use may not reduce mastitis in dairy animals (Barkema et al. 2015; Kayitsinga et al. 2017).

Conclusion

We determined wide distribution of resistance of Gram-positive and Gram-negative bacteria associated with clinical bovine mastitis to Extended Spectrum β Lactamases (ESBL), Cephalosporins, Tetracyclines, Vancomycin and Chloramphenicol. We could demonstrate Vancomycin Resistant Enterococci (VRE), Multiple Drug Resistant Gram-Negative Rods (MDR-GNR), MDR-Pseudomonas (MDR-P) and MDR Acinetobacter (MDR-A) having public health concerns. The ESBLs and cephalosporins resistant *S. aureus* isolates developed marked resistance to vancomycin. Wide spread of resistance among *Streptococcus uberis* against ESBLs and cephalosporins, widely used antibiotics in bovine mastitis, was documented. MDR transfer from non-pathogens to emerging foodborne and established mastitis pathogens could be potential problem to dairy industry as well as public health.

Declarations

Funding :

This work did not receive any grant.

Conflict of Interest :

None

Ethical approval :

Invasive procedure and animal experimentation were not conducted.

Consent to participate :

Consents were obtained from animal owners.

Authors' contributions :

SPA contributed to conception, design and implementation of the work, reviewed and edited the initial draft of the manuscript. MBK provided technical laboratory support, wrote the initial draft of the manuscript.

Availability of Data and Materials:

All data generated and analysed during this study are included in this article.

Consent to publish :

All authors give consent for publication.

References

1. Akhoun ZA, Peer FU, Sofi KA (2012) Study on in vitro sensitivity of bacterial cultures from clinical mastitic milk to few anti-bacterial agents. *Indian Journal of Animal Research*, 46: 404-406
2. Alvarez-Uria G, Gandra S, Mandal S, Laxminarayan R (2018) Global forecast of antimicrobial resistance in invasive isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Int J Infect Dis* 68: 50–53
3. Awad IN, El-Metwally FVM, Abd-El-Moaty AM, Magdy AS (2017) Resistant gene of *Pseudomonas aeruginosa* in mastitic cattle, Biochemical and Immunological Parameters. *World Vet J* 7(1): 05-13
4. Awandkar SP, Khode NV, Sardar VM, Mendhe MS, Kulkarni MB (2009) Prevalence and current antibiogram trend of mastitic agents in Udgir and its vicinity, Maharashtra State, India. *International Journal of Dairy Science* 4(3): 117-122
5. Bandyopadhyay S, Samanta I, Bhattacharyya D, Nanda PK, Kar D, Chowdhury J, Dandapat P, Das AK, Batul N, Mondal B, Dutta TK, Das G, Das BC, Naskar S, Bandyopadhyay UK, Das SC, Bandyopadhyay S (2015) Co-infection of methicillin-resistant *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* and extended spectrum β -lactamase producing *Escherichia coli* in bovine mastitis - three cases reported from India. *Vet Q* 35: 56-61
6. Barkema HW, von Keyserlingk MAG, Kastelic JP, Lam TJGM, Luby C, Roy JP, LeBlanc SJ, Keefe GP, Kelton DF (2015) Invited review: Changes in the dairy industry affecting dairy health and welfare. *J Dairy Sci* 98:7426–7445
7. Bernal-Rosa, Y, Osorio-Muñoz K, Torres-García O (2015) *Pseudomonas aeruginosa*: an emerging nosocomial trouble in veterinary. *Rev. MVZ Córdoba* 20(Supl): 4937-4946

8. Biswal I, Arora BS, Kasana DN (2014) Incidence of multidrug resistant *Pseudomonas aeruginosa* isolated from burn patients and environment of teaching institution. *J. Clin. Diagn. Res.* 8(5): 26-29
9. Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY, Calavas D (2010) Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne Pathog Dis* 7: 479–87
10. Bradley AJ (2012) Bovine mastitis: an evolving disease. *Vet J* 164:116–28
11. Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS, Kumanan K, Vairamuthu S, Ramesh S (2014) Pattern of antibiotic resistant mastitis in dairy cows. *Veterinary World* 7(6): 389-394
12. Clinical Laboratory Standards Institute (2019) Performance Standards for Antimicrobial Susceptibility Testing M100 (29th ed.). Wayne, P.A. USA
13. Constable PD, Hinchcliff KW, Done SH, Grünberg W (2017) Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats (11th ed.) Diseases of the mammary gland. St Louis, MO: Elsevier Ltd. pp. 1904–1996
14. Das A, Guha C, Biswas U, Jana PS, Chatterjee A, Samanta I (2017) Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal. *Veterinary World.* 10(5): 517-520
15. Gao J, Herman WB, Limei Z, Gang L, Zhaoju D, Lingjie C, Ruixue S, Shiyao Z, Jiaqi Z, John PK, Bo H (2017) Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. *J Dairy Sci* 100: 4797–4806
16. Ghatak S, Singha A, Sen A, Guha C, Ahuja A, Bhattacharjee U, Das S, Pradhan NR, Puro K, Jana C, Dey TK, Prashantkumar KL, Das A, Shakuntala I, Biswas U, Jana PS (2013) Detection of New Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in *Escherichia coli* isolated from mastitic milk samples. *Transbound Emer Dis* 60: 385-389
17. Gundogan N, Avci E (2014) Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *Int J Dairy Technol* 67: 562–569
18. Gurung N, Ray S, Bose S, Rai V (1993) A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond", *BioMed Research International*, vol. 2013, Article ID 329121 <https://doi.org/10.1155/2013/329121>
19. Hendriksen RS, Mevius DJ, Schroeter A, Teale C, Meunier D, Butaye P, Franco A, Utinane A, Amado A, Moreno M, Greko C, Stark K, Berghold C, Myllyniemi AL, Wasyl D, Sunde M, Aarestrup FM (2008) Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004. *Acta Vet Scand* 50: 28
20. Hinthong W, Natapol P, Sirijan S, Suphang K, Shutipen B, Nitat S, Wanpen C, Pisinee A, Nitaya I (2017) Detection and drug resistance profile of *Escherichia coli* from subclinical mastitis cows and water supply in dairy farms in Saraburi Province, Thailand. *PeerJ.* <https://doi.org/10.7717/peerj.3431>
21. Hirakawa Y, Sasak, H, Kawamoto E, Ishikawa H, Matsumoto T, Aoyama N (2010) Prevalence and analysis of *Pseudomonas aeruginosa* in chinchillas. *BMC Vet Res* 6: 52

22. Hogeveen H, Huijps K, Lam T (2011) Economic aspects of mastitis: new developments. *NZ Vet J* 59: 16–23
23. Jahan M, Rahman M, Parvej MS, Chowdhury SMZH, Haque ME, Talukder MAK, Ahmed S (2015) Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. *J Adv Vet Anim Res* 2: 49
24. Kalmus P, Aasmae B, Karssin A, Orro T, Kask K (2011) Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta Ve. Scand* 53: 4
25. Kateete DP, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML (2013) Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS One* 8: e63413
26. Kayitsinga J, Schewe RL, Contreras GA, Erskine RJ (2017) Antimicrobial treatment of clinical mastitis in the eastern United States: The influence of dairy farmers' mastitis management and treatment behaviour and attitudes. *J Dairy Sci* 100: 1388–1407
27. Kotwal A, Biswas D, Kakati B, Singh M (2016) ESBL and MBL in Cefepime Resistant *Pseudomonas aeruginosa*: An Update from a Rural Area in Northern India. *Journal of Clinical and Diagnostic Research* <https://doi.org/10.7860/JCDR/2016/18016.7612>
28. Kumar R, Yadav BR, Singh RS (2010) Genetic Determinants of antibiotic resistance in isolates from milk of mastitic crossbred cattle. *Current Microbiology* 60: 379–386
29. Lan T, Huimin L, Lu M, Mengru X, Lei D, Mei G, Jiaqi W, Nan Z (2020) Antimicrobial susceptibility, phylotypes, and virulence genes of *Escherichia coli* from clinical bovine mastitis in five provinces of China. *Food and Agricultural Immunology* 31(1): 406-423
30. Machuca J, Recacha E, Briaes A, Diazdealba P, Blazquez J, Pascual A (2017) Cellular response to ciprofloxacin in low-level quinolone-resistant *Escherichia coli*. *Front Microbiol* 8:1370
31. McMurry L, Levy S (2000) Tetracycline resistance in gram-positive bacteria. *Gram-positive pathogens*. ASM Press Washington DC pp. 660-77
32. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y (2007) *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. *Clin Microbiol Infect* 13(6): 560-578
33. Minst K, Märtilbauer E, Miller T, Meyer C (2012) Streptococcus species isolated from mastitis milk samples in Germany and their resistance to antimicrobial agents. *J Dairy Sci* 95: 6957–6962
34. Nam HM, Lim SK, Kim JM, Joo YS, Jang KC, Jung SC (2010) In vitro activities of antimicrobials against six important species of gram-negative bacteria isolated from raw milk samples in Korea. *Foodborne Pathog Dis* 7: 221–224
35. Nobrega DB, Naushad S, Naqvi SA, Condas LAZ, Saini V, Kastelic JP, Luby, C, De Buck J, Barkema HW (2018) Prevalence and genetic basis of antimicrobial resistance in non-aureus Staphylococci isolated from Canadian dairy herds. *Front Microbiol* 9: 256
36. Pitkala A, Salmikivi L, Bredbacka P, Myllyniemi AL, Koskinen MT (2004) Comparison of tests for detection of beta-lactamase-producing Staphylococci. *J Clin Microbiol* 45: 2031-2033

37. Ranjan R, Gupta MK, Singh S, Kumar S (2010) Current trend of drug sensitivity in bovine mastitis. *Veterinary World* 3: 17-20
38. Sadashiv SO, Kaliwal BB (2014) Isolation, characterization and antibiotic resistance of *Bacillus spp.* from bovine mastitis in the region of north Karnataka, India. *Int J Curr Microbiol App Sci* 3(4): 360-373
39. Saini V, McClure JT, Leger D, Keefe GP, Scholl DT, Morck DW, Barkema HW (2012) Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. *J Dairy Sci* 95: 4319–4332
40. Salauddin M, Akter MR, Hossain MK, Rahman MM (2019) Isolation of multi-drug resistant *Klebsiella* sp. from bovine mastitis samples in Rangpur, Bangladesh. *J Adv Vet Anim Res* 6(3): 362–365
41. Salauddin M, Mir RA, Khaled Hossain M, Nazir KHM, Noreddin A, Mohamed EZ (2020) Molecular Detection of Multidrug Resistant *Staphylococcus aureus* Isolated from Bovine Mastitis Milk in Bangladesh. *Vet Sci* 7: 36
42. Salih RRM, Gibreel HH (2019) Gentamicin is the best antibiotic in treatment all types of bovine mastitis caused by bacteria. *Agriculture and Forestry Journal* 3(1): 46-49
43. Schukken Y, Chuff M, Moroni P, Gurjar A, Santisteban C, Welcome F, Zadoks RN (2012) The “other” gram-negative bacteria in mastitis: *Klebsiella*, *Serratia*, and more. *Vet Clin North Am Food Anim Pract* 28: 239-256
44. Weisblum B (1995) Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother* 39: 577–585
45. Yan, F, Shidong Z, Xiaofei S, Ling W, Hongsheng L, Xurong W (2018) Characteristics of quinolone-resistant *Escherichia coli* isolated from bovine mastitis in China. *J Dairy Sci* 101: 6244–6252

Figures

Figure 1

MALDI-TOF protein spectra (a) and matching patterns (b) for different representative bacterial isolates obtained from bovine mastitis

Figure 2

Antimicrobial susceptibility patterns of bacterial isolates obtained from bovine mastitis