Bacteria from bovine clinical mastitis showed multiple drug resistance

Sudhakar P. Awandkar1,2 · Mahesh B. Kulkarni1,2 · Narendra V. Khode1,2

Received: 23 March 2021 / Accepted: 20 September 2021 / Published online: 27 September 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract
Mastitis, which often manifests as udder infection in dairy animals, is of great concern as it affects public health and results in heavy economic losses to the dairy industry. A hospital-based cross-sectional study was conducted to determine the culturable bacterial species associated with bovine clinical mastitis and their resistance patterns towards different antimicrobials. The milk samples from cows suffering from clinical mastitis during monsoon season were investigated. The prevalence of clinical mastitis was significantly high in Holstein–Friesian crossbred cows, followed by Jersey crossbred, Red Kandhari and Deoni. Significantly high prevalence was observed during 4th to 6th months of lactation. A total of 110 bacterial isolates belonging to 14 different genera were isolated and identified. Aminoglycosides and quinolones were found to be the most effective antibiotics. Vancomycin resistant penicillinase producing Gram positive bacteria were demonstrated. Gram negative bacteria resistant to extended spectrum β lactamases, cephalosporins, tetracyclines, vancomycin and chloramphenicol as well as vancomycin resistant enterococci, multiple drug resistant (MDR) gram negative rods, MDR Pseudomonas and MDR Acinetobacter were found. Widespread resistance of Streptococcus uberis towards cephalosporins was documented. Variable MDR patterns were recorded within a single species. MDR transfer from non-pathogens to emerging foodborne and established mastitis pathogens could be a potential problem to the dairy industry as well as to public health.

Keywords Multiple drug resistance · Clinical mastitis · Bovine · India

Introduction
Bovine mastitis is professed to be the leading cause of impaired productivity and health issues in dairy animals (Hogeveen et al. 2011). Mastitis is a complex, multi-factorial illness caused by a variety of pathogens, including bacteria, viruses and fungi. The occurrence of the disease may vary depending upon the animals affected, pathogens involved and the environment (Constable et al. 2017). Several bacterial agents associated with bovine mastitis are commonly isolated from the dairy environment (Bradley 2012).

Streptococcus, Staphylococcus and Corynebacterium spp. are potential bacteria capable of causing clinical as well as subclinical mastitis. In addition, bacteria of environmental aetiology, including Escherichia, Acinetobacter, Pasteurella, Pseudomonas, Klebsiella, Arthrobacter, Bacillus, Enterobacter, Enterococcus and Serratia are involved in aggravated conditions. These organisms often carry antimicrobial resistance (AMR) genes and are developing multiple drug resistance (MDR) to popular antimicrobial agents used in treating mastitis (Weisblum 1995; McMurry and Levy 2000; Botrel et al. 2010). Not responsible use of antibiotics leads to mutations in bacteria, allowing them to survive and making treatment a complicated and costly affair. However, bacterial isolates associated with bovine mastitis are dramatically susceptible to gentamicin (Awandkar et al. 2009; Salih and Gibreel 2019). Only sporadic literature is available on MDR in Indian dairy animals. Therefore, this study was planned to decipher the antimicrobial resistance profile of bacterial pathogens linked to clinical bovine mastitis in dairy cows.

Materials and methods

Study design, milk sampling and screening
A hospital-based cross-sectional study was performed on lactating cows that reported to the Veterinary Clinical
Complex, Udgir, during the monsoon season (2018–2200). The cases were documented from seven districts (Nanded, Latur, Osmanabad, Beed, Solapur, Bidar and Gulbarga) of Western India. The sample size was estimated using the formula $n = (Za/2)^2 \times p(1-p)/d^2$ for calculating the single population proportion at 95% confidence interval ($Za/2 = 1.96$) and 5% margin of error by considering a 10% prevalence rate (Joshi and Gokhale 2006). A total of 1382 lactating cows belonging to four breeds (Deoni: 443, Red Kandhari: 92, Jersey crossbred: 248, Holstein–Friesian [HF] crossbred: 599) and three lactation stages (0–3 months: 447, 4–6 months: 513, 7–9 months: 422) were recruited for the study. Out of these, 272 lactating cows exhibiting swelling of the udder, udder pain, swollen teat and visible changes in the milk were considered for sampling. The udder and teats were washed with 1% KMnO4 and wiped with clean and smooth cloth. The first four strips were discarded, and 10 mL of milk sample was aseptically collected in a sterile disposable vial. The samples were brought to the Microbiology Laboratory in ice-cooled containers. The samples were screened using the California mastitis test (CMT), and were processed within 24 h of collection.

**Bacterial isolation**

Each positive sample (100 μL) was inoculated by streaking on 5% sheep blood agar and incubated aerobically for 24–48 h at 37 °C. The colonies of the suspected pathogens were further subcultured on brain heart infusion agar for lyase preparation and antimicrobial susceptibility testing.

**Bacterial identification**

The bacterial isolates were identified by observing the nature of growth, cultural characteristics, Gram staining reaction and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis (Bruker Daltonics, Bremen, Germany).

The protein was extracted from the 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, 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 protein 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 a-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and air dried. Mass spectra were then acquired using the Microflex MALDI-TOF MS. MALDI BioTyper 2.0 software was employed for spectral analysis and comparison with the MALDI BioTyper database. A score of ≥ 2 was considered valid for species-level identification, while that between 1.7 and 2 was considered valid for genus-level identification. Score ≤ 1.7 was considered invalid. The staphylococci were further subjected to coagulase test as per the standard protocol.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing of all the bacterial isolates was performed by the Kirby–Bauer agar disc diffusion method using Mueller Hinton agar (MHA, HiMedia, India) in four replicates. The CLSI guidelines were followed (Clinical Laboratory Standards Institute, 2020). Thirteen commercially available antibiotic discs (HiMedia, 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), gentamicin (10 mcg), oxytetracycline (30 mcg), penicillin (10 units) and vancomycin (30 mcg) were utilized for 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 4–10 h until the turbidity reached 0.5 McFarland’s standard. The inoculum was spread onto the entire surface of a sterile MHA medium with sterile cotton swabs. The plate was incubated at room temperature for 10 min before placing the antibiotic discs. The plates were then incubated at 37 °C for 16 h. The average of at least two perpendicular diameters of growth inhibition zones was recorded. Resistance of *Staphylococcus aureus* towards β lactam antibiotics was determined by including penicillin (10 units) discs. Resistance of *Staphylococcus aureus* and *Enterococcus faecalis* to glycopeptides was determined by including vancomycin (30 mcg) discs. Cefotaxime (30 mcg) and ceftriaxone–tazobactam (30/10 mcg) discs were used to determine extended spectrum β lactamase (ESBL) producing *Escherichia coli*.

**Statistical analysis**

The prevalence of bovine clinical mastitis and antibiotic resistance was expressed in percentage. The breed and lactation stage wise prevalence of clinical mastitis was analysed by using binary logistic regression model in IBM SPSS-20.
software package. The results of antibiotic resistance were analysed for statistical significance by applying $\chi^2$ test. Non-susceptibility to at least one antibiotic in three or more antibiotic groups was considered as MDR.

**Results**

Totally, 272 milk samples (19.68%) were found to be positive upon clinical inspection as per CMT. The overall logistic regression model was significant indicating effect of breed and lactation stage on the clinical mastitis. The predicted odds of having clinical mastitis was 0.245 which was not very high. The breed (odds ratio 1.193, 95% confidence interval 1.074 to 1.327) and lactation stage (odds ratio 1.246, 95% confidence interval 1.052 to 1.475) were highly significant ($p < 0.01$) for clinical mastitis.

**Breed-wise prevalence of clinical mastitis**

The results revealed that there was highly significant ($p = 0.001$) change in the breed-wise prevalence of clinical mastitis. Within breed effect was significant only for HF cross bred cows, however, for others, the within breed differences were not observed. Between the breeds, the odds of clinical mastitis were not much different when compared to HF cross bred cows. The highest prevalence was recorded in HF crossbred cows, followed by Jersey crossbred, Red Kandhari and Deoni cows (Table 1).

**Lactation stage-wise prevalence of clinical mastitis**

The lactation stage wise occurrence of clinical mastitis was highly significant ($p = 0.011$). When the prevalence of clinical mastitis during 7th to 9th months of lactation was compared with the prevalence during 0th to 3rd months and 4th to 6th months, the odds of occurrence of clinical mastitis was higher in 4th to 6th months of lactation as compared to other lactation stages (Table 2).

**Bacterial isolates obtained from cows with clinical mastitis**

A total of 110 bacterial isolates belonging to 14 different genera were isolated and identified (Table 3 and Fig. 1). Among them, non-$\text{aureus}$ Staphylococci (NAS, 26.36%) emerged as the principal pathogen associated with bovine mastitis, followed by $\text{Staphylococcus aureus}$ (9.09%), $\text{Escherichia coli}$ (8.18%), $\text{Bacillus spp.}$ (7.27%), $\text{Streptococcus uberis}$, $\text{Pseudomonas aeruginosa}$ and $\text{Enterococcus faecalis}$ (6.36% each), $\text{Acinetobacter baumannii}$ and $\text{Enterobacter}$ spp. (4.55% each), $\text{Klebsiella pneumoniae}$ (2.73%) and $\text{Corynebacterium}$ spp. (1.82%). The other bacteria included $\text{Pseudomonas putida}$, $\text{Escherichia hermanii}$, $\text{Lactococcus spp.}$, $\text{Pasteurella}$ spp., $\text{Enterococcus}$ spp., $\text{Acinetobacter pittii}$, $\text{Serratia marcescens}$ and $\text{Arthrobacter arilaitensis}$ (16.36%).

| Table 1 | Breed wise prevalence of bovine clinical mastitis |
|---------|-------------------------------------------------|
| Breed   | Total number examined | Positive | Percentage | Wald | Significance | Exp(B) (CI) |
| Deoni   | 443                  | 66       | 14.90     | 9.898 | 0.002       | 0.592 (0.427, 0.821) |
| Red Kandhari | 92       | 15       | 16.30     | 1.903 | 0.168       | 0.660 (0.366, 1.191) |
| Jersey Crossbred | 248 | 55       | 22.18     | 0.027 | 0.869       | 0.970 (0.678, 1.389) |
| HF crossbred | 599     | 136      | 22.70     | 11.486 | 0.009       |                         |
| Total   | 1382                | 272      | 19.68     |       |             |                         |

$Exp(B)$ odds ratio, $CI$ confidence interval 95% for Exp(B)

| Table 2 | Lactation stage wise prevalence of bovine clinical mastitis |
|---------|----------------------------------------------------------|
| Lactation stage | Total number examined | Positive | Percentage | Wald | Significance | Exp(B) (CI) |
| 0–3 Months      | 447               | 58       | 12.98     | 7.079 | 0.008       | 0.608 (0.421, 0.877) |
| 4–6 Months      | 513               | 131      | 25.54     | 4.535 | 0.033       | 1.405 (1.027, 1.921) |
| 7–9 Months      | 422               | 83       | 19.67     | 23.314 | 0.000 |
| Total           | 1382              | 272      | 19.68     |       |             |                         |

$Exp(B)$ odds ratio, $CI$ confidence interval 95% for Exp(B)
Antibiotic sensitivity

The species-wise results of the antibiotic sensitivity test for *Staphylococcus aureus*, NAS, *Streptococcus uberis*, *Enterococcus faecalis*, Bacillus spp., *Escherichia coli*, *Enterobacter* spp., *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are presented in Fig. 2.

Most of the *Staphylococcus aureus* isolates were found to exhibit MDR, and different patterns were recorded. The isolates (90%) showed significantly high resistance (p < 0.001) towards β lactam antibiotics as compared to amoxicillin–sulbactam (30%). Of these isolates, 70% were also found to be resistant towards vancomycin, followed by amikacin (60%) and oxytetracycline (50%). The isolates were significantly less resistant (p < 0.001) towards gentamicin and ciprofloxacin (10% each), followed by chloramphenicol, doxycycline, azithromycin (20% each), amoxicillin–sulbactam (30%) and enrofloxacin (40%).

Almost all NAS isolates showed resistance towards penicillin. Significantly less resistance (p < 0.001) was

---

**Table 3** Bacterial isolates from bovine clinical mastitis

| SN | Genera       | Bacterial isolates | Total no | MALDI-TOF score | Percentage |
|----|--------------|--------------------|----------|-----------------|------------|
| 1  | *Acinetobacter* | *Acinetobacter baumannii* | 5        | 2.213           | 5.50       |
|    |              | *Acinetobacter pittii* | 1        | 2.115           |            |
| 2  | *Arthrobacter* | *Arthrobacter arilaitensis* | 1        | 2.421           | 0.92       |
| 3  | *Bacillus*    | *Bacillus cereus* | 5        | 2.402           | 7.34       |
|    |              | *Bacillus spp.* | 2        | 1.842           |            |
|    |              | *Bacillus subtilis* | 1        | 2.087           |            |
| 4  | *Corynebacterium* | *Corynebacterium casei* | 1        | 2.588           | 1.83       |
|    |              | *Corynebacterium spp.* | 1        | 1.986           |            |
| 5  | *Enterobacter* | *Enterobacter asburiae* | 1        | 2.379           | 4.59       |
|    |              | *Enterobacter aerogenes* | 1        | 2.314           |            |
|    |              | *Enterobacter cloacae* | 3        | 2.243           |            |
| 6  | *Enterococcus* | *Enterococcus casseliflavus* | 2        | 2.245           | 10.09      |
|    |              | *Enterococcus faecalis* | 3        | 2.440           |            |
|    |              | *Enterococcus faecium* | 1        | 2.308           |            |
|    |              | *Enterococcus gallinarum* | 5        | 2.213           |            |
| 7  | *Escherichia* | *Escherichia coli* | 9        | 2.475           | 9.17       |
|    |              | *Escherichia hermannii* | 1        | 2.441           |            |
| 8  | *Klebsiella*  | *Klebsiella pneumoniae* | 3        | 2.483           | 2.75       |
| 9  | *Lactococcus* | *Lactococcus lactis* | 2        | 2.475           | 1.83       |
| 10 | *Pasteurella* | *Pasteurella spp.* | 1        | 1.969           | 1.83       |
|    |              | *Pasteurella multocida* | 1        | 2.443           |            |
| 11 | *Pseudomonas* | *Pseudomonas aeruginosa* | 8        | 2.445           | 10.09      |
|    |              | *Pseudomonas mosselii* | 1        | 2.440           |            |
|    |              | *Pseudomonas putida* | 2        | 2.208           |            |
| 12 | *Serratia*   | *Serratia marcescens* | 2        | 2.253           | 1.83       |
| 13 | *Staphylococcus* | *Staph. aureus* | 10       | 2.485           | 35.78      |
|    |              | *Staph. arlettae* | 10       | 2.341           |            |
|    |              | *Staph. chromogenes* | 2        | 2.488           |            |
|    |              | *Staph. cohnii sp. urealyticus* | 2        | 2.294           |            |
|    |              | *Staph. epidermidis* | 2        | 2.341           |            |
|    |              | *Staph. haemolyticus* | 3        | 2.445           |            |
|    |              | *Staph. hominis* | 1        | 2.475           |            |
|    |              | *Staph. pseudoepidemidis* | 1        | 2.314           |            |
|    |              | *Staph. saprophyticus* | 2        | 2.308           |            |
|    |              | *Staph. sciuri* | 4        | 2.214           |            |
|    |              | *Staph. simulans* | 2        | 2.336           |            |
| 14 | *Streptococcus* | *Strep. uberis* | 6        | 2.389           | 7.34       |
|    |              | *Strep. spp.* | 2        | 1.795           |            |
recorded for ciprofloxacin (96.55%), followed by doxycycline (89.66%), chloramphenicol (86.21%) and vancomycin (79.31%). Non-significant less resistance was found for oxytetracycline (62.07%). Aminoglycosides were the most effective antibiotics against NAS as there was no resistance.

*Streptococcus uberis* depicted resistance towards all the antibiotics used. The resistance towards β-lactam antibiotics (57.14%–100%), oxytetracycline (100%), vancomycin, enrofloxacin, chloramphenicol and doxycycline (57.14% each) were non-significant.

*Enterococcus faecalis* also displayed resistance towards all the antibiotics studied. Penicillin, ceftriaxone–tazobactam and cefotaxime were found to be totally ineffective against *Enterobacter faecalis* isolates from the milk samples. Most of the isolates showed highly significant resistance (*p* < 0.001) towards vancomycin (81.82%). Non-significant resistance was recorded for amoxicillin–sulbactam and amikacin (63.64% each), enrofloxacin and oxytetracycline (45.45% each) and doxycycline (36.36%). Significantly less resistance (*p* = 0.001) was documented for gentamicin (9.09%) and ciprofloxacin (18.18%).

The Bacillus isolates did not exhibit resistance towards amikacin, gentamicin, enrofloxacin and ciprofloxacin, while almost complete resistance was recorded towards β-lactam antibiotics such as penicillin and cefotaxime (100% each), followed by significant resistance (*p* < 0.005) towards ceftriaxone–tazobactam (75%) and amoxicillin–sulbactam (87.50%). The resistance towards oxytetracycline (62.50%) and chloramphenicol (37.50%) were non-significant. Significant susceptibility (*p* < 0.005) was recorded for doxycycline.

More than 30% *Escherichia coli* isolates showed resistance towards all the antibiotics except gentamicin. All the isolates presented resistance towards penicillin, while significantly high resistance (*p* < 0.001) was recoded for cefotaxime (90%), oxytetracycline and ceftriaxone–tazobactam (80% each). Non-significant resistance was found for amoxicillin–sulbactam (70%), amikacin (60%), doxycycline (50%), enrofloxacin, ciprofloxacin (40% each) and chloramphenicol (30%).

The *Enterobacter* isolates depicted 100% sensitivity towards enrofloxacin, ciprofloxacin and chloramphenicol and complete resistance towards penicillin and amoxicillin–sulbactam. Non-significant resistance was recorded
against oxytetracycline, doxycycline, amikacin (80% each), cefotaxime, ceftriaxone–tazobactam (60% each) and gentamicin (20%). Penicillin and amoxicillin–sulbactam were totally ineffective against Enterobacter isolates obtained from the milk samples.

Acinetobacter baumannii showed almost complete resistance towards all the β lactam antibiotics and oxytetracycline. Non-significant resistance was found against amikacin. The isolates were significantly less resistant (p < 0.001) towards doxycycline, gentamicin, enrofloxacin and ciprofloxacin (16.67% each).

Pseudomonas aeruginosa demonstrated significantly high resistance (p < 0.001) towards oxytetracycline and doxycycline (90.91% each) and significant resistance (p < 0.001) towards amikacin (27.27%). These isolates were found to be completely sensitive towards gentamicin. Non-significant less resistance was noted for enrofloxacin and ciprofloxacin (9.09% each). The isolates were significantly sensitive towards aminoglycosides and quinolones.

Klebsiella isolates possessed complete resistance towards vancomycin, lincomycin, amikacin, ciprofloxacin, cefotaxime, erythromycin, penicillin and amoxicillin–sulbactam. These isolates were inferred to be sensitive towards chloramphenicol and enrofloxacin only. Corynebacterium isolates showed sensitivity towards amoxicillin–sulbactam, azithromycin, ceftriaxone–tazobactam, amikacin,
gentamicin, doxycycline, enrofloxacin and vancomycin, while _Lactococcus_ isolates displayed complete resistance towards penicillin only. _Pasteurella_ isolates exhibited total resistance towards oxytetracycline, doxycycline, amikacin, enrofloxacin, ciprofloxacin, cefotaxime, ceftriaxone–tazo-bactam, penicillin, amoxicillin–subactam and vancomycin and sensitivity towards chloramphenicol, azithromycin and gentamicin. _Serratia_ spp. were resistant towards vancomycin, oxytetracycline, doxycycline, amikacin, cefotaxime, ceftriaxone–tazo-bactam, erythromycin, penicillin and amoxicillin–subactam and sensitive towards azithromycin, gentamicin, enrofloxacin and ciprofloxacin.

**Multiple drug resistance**

The MDR patterns of Gram positive and Gram-negative bacteria associated with bovine clinical mastitis were evaluated (Tables 4 and 5).

In _Staphylococcus aureus_, tetracycline-aminoglycosides-quinolones-cephalosporin-macrolide resistance pattern was found in one isolate. Glycopeptides–tetracycline-quinolones-cephalosporin-β lactam and chloramphenicol-glycopeptides-cephalosporin-β lactam resistance patterns were recorded in two isolates each. Three isolates depicted resistance towards glycopeptides–tetracycline-cephalosporin-β lactam, glycopeptides-quinolones-cephalosporin-β lactam and glycopeptides-cephalosporin-β lactam groups of antibiotics. Four isolates showed glycopeptides-aminoglycosides-cephalosporin-β lactam and tetracycline-cephalosporin-β lactam resistance patterns.

The MDR patterns of _Streptococcus uberis_ observed for chloramphenicol-glycopeptides–tetracycline-quinolones-cephalosporin-β lactam and cephalosporin–macrolides-β lactam were restricted to one isolate each. However, two isolates showed chloramphenicol-glycopeptides–tetracycline-cephalosporin-β lactam and tetracycline-aminoglycosides-quinolones-cephalosporin-β lactam resistance patterns. Glycopeptides–tetracycline-cephalosporin-β lactam resistance pattern was found in three isolates. Four MDR isolates demonstrated tetracycline-quinolones-cephalosporin-β lactam resistance pattern.

At least one _Enterococcus faecalis_ isolate presented MDR towards glycopeptides–tetracycline-quinolones-cephalosporin-β lactam, glycopeptides–tetracycline-cephalosporin-β lactam and glycopeptides–aminoglycosides-quinolones-cephalosporin-β lactam. Glycopeptides-cephalosporin-β lactam and tetracycline-aminoglycosides-cephalosporin-β lactam resistance patterns were recorded in two isolates each. Similarly, three isolates revealed tetracycline/macrolides/glycopeptide-cephalosporin-β lactam resistance pattern.

_Bacillus cereus_ isolates (one each) showed MDR towards eight antibiotics distributed over four and five antibiotic groups. Most of the isolates showed resistance towards chloramphenicol–glycopeptide–tetracycline-cephalosporins–β lactams.

The MDR patterns recorded for _Escherichia coli_ isolates were chloramphenicol–tetracycline-quinolones-cephalosporin-β lactam, tetracycline-quinolones-cephalosporin-β lactams and aminoglycosides-cephalosporin-β lactams. _Enterobacter_ isolates showed common MDR patterns towards antimicrobials from the tetracycline–aminoglycosides-cephalosporin-β lactam group. _Acinetobacter baumannii_, an udder inhabitant, demonstrated considerable MDR with variable patterns. Tetracycline-aminoglycosides/quinolones-cephalosporin-β lactam and tetracycline-cephalosporin-β lactam MDR patterns were recorded in these isolates.

**Discussion**

Our findings revealed the significant prevalence of clinical mastitis in HF crossbred followed by Jersey crossbred cows. The Red Kandhari and Deoni cows were comparatively less susceptible to clinical mastitis. Significantly higher prevalence of clinical mastitis in cross bred cows could be due to higher milk productivity and the associated stress.

The significantly high prevalence of clinical mastitis was observed during 4th to 6th months of lactation. This lactation stage was more prone for occurrence of clinical mastitis. The higher prevalence during this stage could be ascribed to the physiological stress of high milk production and alterations in homeostasis (Joshi and Gokhale 2006).

In clinical mastitis, a comparable distribution of _Staphylococcus_ spp., _Pseudomonas_ spp., _Streptococcus_ spp., _Pasteurella_ spp., _Enterobacter_ spp., _Klebsiella_ spp. and _Corynebacterium_ spp. has been reported earlier (Das et al. 2017). When compared with certain previous reports, we have reported higher distribution of _Pseudomonas_ spp. and _Enterococcus_ spp. and less but variable distribution of _Staphylococcus_ spp., _Escherichia_ spp. and _Klebsiella_ spp. (Gundogan and Avci 2014; Jahan et al. 2015; Gao et al. 2017; Salauddin et al. 2020). Other than _Staphylococcus_ spp., isolates such as _Pseudomonas_ spp., _Enterococcus_ spp., _Escherichia_ spp., _Streptococcus_ spp. and _Bacillus_ spp. emerged as the major bacteria associated with bovine mastitis in the study area. We further isolated _Pseudomonas aeruginosa_ and _Acinetobacter baumannii_, which are of public health significance. Various researchers have reported low isolation rates for _Acinetobacter baumannii_ in milk samples, which is in accordance with the findings of the present study (Gurung et al. 1993; Nam et al. 2010). Variations in the distribution patterns of mastitis bacteria may indicate their geographical dissemination, health status and biosecurity practices of the study area (Salauddin et al. 2019).
Jahan et al. (2015) and Salauddin et al. (2020) documented antibiotic resistance in *Staphylococcus aureus* comparable to the results of the present investigation. Past studies have reported the isolation of antibiotic resistant *Bacillus* spp. with 91.40% resistance towards penicillin, followed by ampicillin (50.67%), ceftriaxone (35.29%), erythromycin (20.36%) and azithromycin (5.42%) from animals with bovine mastitis. These findings agree with the results of the present investigation (Sadashiv and Kaliwal 2014). All the *Escherichia coli* isolates were sensitive to gentamicin. On the contrary, earlier reports have suggested that majority of the *Escherichia coli* isolates from animals with bovine mastitis were resistant to gentamicin and susceptible to amoxicillin and ciprofloxacin (Yang et al. 2018; Lan et al. 2020).

| SN | Isolates     | n | MDR patterns                                                                 |
|----|--------------|---|------------------------------------------------------------------------------|
| 1  | *Staph. aureus* | 1 | OTC, DOX, AMK, GEN, ENR, CIP, CFO, CFT, AZI                                 |
|    |              |   | VAN, OTC, ENR, CFO, CFT, PEN, AMS                                           |
|    |              |   | VAN, OTC, CFO, CFT, PEN, AMS                                                |
|    |              | 2 | CHL, VAN, CFO, CFT, PEN                                                     |
|    |              |   | VAN, AMK, CFO, CFT, PEN                                                     |
|    |              |   | OTC, CFO, CFT, PEN                                                          |
|    |              |   | OTC, CFO, CFT, PEN                                                          |
|    |              |   | VAN, CFO, CFT, PEN                                                          |
|    | *Staph. arlettae* | 1 | OTC, DOX, CFO, CFT, AZI, PEN                                                 |
|    |              |   | OTC, CFO, CFT, AZI, PEN                                                     |
|    |              |   | CHL, CFO, AZI, PEN                                                          |
|    |              |   | CHL, OTC, CFO, AZI, PEN                                                     |
|    |              | 4 | OTC, AZI, PEN                                                               |
|    |              |   | CFO, CFT, AZI, PEN                                                          |
|    | *Staph. sciuri* | 2 | CHL, OTC, DOX, CFO, CFT, AZI, PEN                                            |
|    | *Strep. uberis* | 1 | CHL, VAN, OTC, CIP, CFO, CFT, PEN, AMS                                       |
|    |              |   | OTC, AMK, ENR, CIP, CFO, CFT, PEN, PEN                                       |
|    |              |   | CHL, VAN, OTC, CFO, PEN                                                     |
|    |              |   | OTC, DOX, ENR, CIP, CFO, PEN                                                |
|    |              |   | OTC, ENR, CIP, CFO, CFT, PEN                                                |
|    |              |   | VAN, OTC, CFO, PEN                                                          |
|    |              | 1 | CFO, CFT, AZI, PEN                                                          |
|    |              |   | OTC, ENR, CIP, CFO, PEN                                                     |
|    |              |   | OTC, AZI, PEN                                                               |
|    |              |   | CHL, OTC, CFO, CFT, PEN                                                     |
|    | *Corynebacterium spp.* | 3 | CHL, OTC, CIP, ERY, PEN                                                    |
|    | *Enterococcus faecalis* | 4 | VAN, OTC, DOX, ENR, CFO, CFT, PEN, AMS                                    |
|    |              |   | VAN, OTC, DOX, CFO, CFT, PEN, AMS                                           |
|    |              |   | VAN, GEN, ENR, CFO, CFT, PEN                                                |
|    |              |   | VAN, CFO, CFT, PEN                                                          |
|    |              | 2 | OTC, DOX, AMK, CFO, CFT, PEN, AMS                                           |
|    |              |   | OTC, DOX, CFO, CFT, PEN                                                     |
|    |              | 3 | OTC, DOX, CFO, CFT, PEN                                                     |
|    |              |   | VAN, CFO, CFT, PEN                                                          |
|    |              | 3 | ENR, CFO, CFT, PEN                                                          |
|    | *Bacillus cereus* | 5 | CHL, OTC, DOX, CFO, CFT, PEN, AMS                                           |
|    |              |   | CHL, DOX, CFO, CFT, AZI, PEN                                                |
|    |              |   | CHL, OTC, CFO, CFT, PEN                                                     |
|    |              | 3 | OTC, DOX, CFO, CFT, PEN                                                     |
|    |              | 2 | OTC, DOX, CFO, CFT, PEN                                                     |
|    |              | 5 | OTC, CFO, CFT, PEN                                                          |
|    |              | 3 | CFO, CFT, PEN                                                               |
|    | *Lactococcus lactis* | 6 | CHL, LIN, OTC, DOX, CFO, CFT, AZI, PEN                                    |
|    |              |   | VAN, OTC, DOX, CFO, PEN                                                     |

CHL Chloramphenicol, VAN Vancomycin, OTC Oxytetracycline, DOX Doxycycline, AMK Amikacin, GEN Gentamicin, ENR Enrofloxacin, CIP Ciprofloxacin, CFO Cefotaxime, CFT Ceftriaxone-tazobactam, AZI Azithromycin, PEN Penicillin, AMS Amoxicillin-sulbactam
Low preference to gentamicin in veterinary therapy may be the reason behind this high sensitivity.

Complete resistance to β-lactams and the MDR exhibited by *Staphylococcus aureus* involved in bovine mastitis could be attributed to the indiscriminate use of these antibiotics in therapy (Pitkala et al. 2004; Hendriksen et al. 2008; Kalmus et al. 2011). The MDR *Staphylococcus aureus* reported in this study demonstrated resistance towards 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 the organisms have acquired complete resistance towards β-lactam antibiotics. Increasing resistance to vancomycin may have implications on animal and human health.

Earlier reports have divergently suggested the common resistance of *Streptococcus uberis* towards tetracyclines, followed by gentamicin, and the absence of resistance towards penicillin and ampicillin (Minst et al. 2012). Other studies have reported Streptococci with relatively high MDR as well as complete resistance towards penicillin (Schukken et al. 2012) and intermediate resistance towards vancomycin (Kateete et al. 2013; Nobrega et al. 2018). This report suggested that β-lactam-resistant *Streptococcus uberis* has acquired considerable resistance towards vancomycin too, which may lead to health concerns in the near future.

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

Previous reports have also hinted the complete resistance exhibited by *Pseudomonas aeruginosa* against many antibiotics, which is similar to the findings of the current study (Bernal-Rosas et al. 2015). β-lactam antibiotics and cephalosporins have been suggested to be essential components in the treatment of *Pseudomonas aeruginosa* infection (Mesaros et al. 2007). However, the development of high resistance towards these antibiotic groups uncovered in the present report is alarming. Ciprofloxacin remains to be effective, along with gentamicin and enrofloxacin. Similar findings were stated by Hirakawa et al. 2010; Ranjan et al. 2010; Akhoon et al. 2012; Biswal et al. 2014; Kotwal et al. 2016; Awad et al. 2017).

Quinolones are widely used in veterinary and medical healthcare. However, high quinolone resistance in *Escherichia coli* and other Gram-negative bacteria has been identified in the present study. Similar findings have been documented earlier by Machuca et al. (2017). A few reports on the involvement of ESBL producing and tetracycline resistant *Escherichia coli* in bovine mastitis (Ghatak et al. 2013; Bandyopadhyay et al. 2015) support the present findings. Indiscriminate antibiotic use may have contributed to the increased

![Table 5](image-url)

**Table 5** MDR patterns of Gram-negative bacteria associated with bovine clinical mastitis

| SN | Isolates            | n  | MDR patterns                                      |
|----|---------------------|----|--------------------------------------------------|
| 1  | *Escherichia coli*  | 2  | CHL, OTC, DOX, ENR, CIP, CFO, CFT, PEN, AMS       |
| 7  | OTC, DOX, ENR, CIP, CFO, CFT, PEN, AMS |
| 1  | OTC, AMK, CFO, CFT, PEN, AMS                     |
| 5  | AMK, CFO, CFT, PEN, AMS                           |
| 2  | *Escherichia hermannii*                           | 1  | CHL, OTC, DOX, AMK, CFO, CFT, PEN                |
| 3  | AMK, CIP, CFO, AZI, PEN, AMS                      |
| 3  | AMK, CIP, CFO, PEN, AMS                           |
| 2  | *Klebsiella pneumoniae*                           | 1  | OTC, AMK, CIP, CFO, CFT, AZI, PEN, AMS           |
| 3  | AMK, CIP, CFO, AZI, PEN, AMS                      |
| 2  | AMK, CIP, CFO, PEN, AMS                           |
| 3  | AMK, CIP, CFO, PEN, AMS                           |
| 2  | *Pasteurella spp.*                                 | 1  | OTC, DOX, AMK, CIP, CFT, PEN, AMS                 |
| 3  | OTC, DOX, AMK, CIP, CFT, PEN, AMS                  |
| 2  | *Serratia marcescens*                             | 1  | OTC, CIP, CFT, PEN, AMS                           |
| 5  | OTC, AMK, CIP, CFT, PEN, AMS                      |
| 5  | *Acinetobacter baumannii*                         | 1  | OTC, GEN, ENR, CIP, CFT, PEN, AMS                 |
| 2  | OTC, DOX, AMK, CIP, CFT, PEN, AMS                  |
| 4  | OTC, AMK, CIP, CFT, PEN, AMS                      |
| 4  | OTC, CIP, CFT, PEN, AMS                           |
| 6  | *Enterobacter spp.*                               | 2  | OTC, DOX, AMK, CIP, CFT, PEN, AMS                 |

*CHL* Chloramphenicol, *VAN* Vancomycin, *OTC* Oxytetracycline, *DOX* Doxycycline, *AMK* Amikacin, *GEN* Gentamicin, *ENR* Enrofloxacin, *CIP* Ciprofloxacin, *CFO* Cefotaxime, *CFT* Ceftriaxone-tazobactam, *AZI* Azithromycin, *PEN* Penicillin, *AMS* Amoxicillin-sulbactam
prevalence of MDR *Escherichia coli* (Chandrasekaran et al. 2014; Hintong et al. 2017). Moreover, the presence of antimicrobials in the environment of the dairy animals may be the reason for MDR in *Escherichia coli*, which is ubiquitous in the cow’s environment. The ability to produce β-lactamase and penicillin binding proteins might have led to resistance towards the penicillin group of antibiotics (Kumar et al. 2010). Symptomatic therapy maybe helpful in alleviating the clinical symptoms instead of resorting to antimicrobial therapy against MDR *Escherichia coli* (Schukken et al. 2012). The resistance of *Escherichia coli* to β-lactam and cephalosporin group of antibiotics may be due to the production of ESBL. However, resistance towards cephaplospirins owing to the production of AmpC cephalosporinase needs to be explored.

Our results allude the complete resistance of *Klebsiella pneumoniae* towards third generation cephalosporins, which reinforces the views of earlier investigators that these drugs will become ineffective against stabilized populations of *Klebsiella pneumoniae* in most parts of the world by 2030 (Alvarez-Uria et al. 2018). Our findings imply the high prevalence of MDR in *Klebsiella pneumoniae* as reported earlier (Schukken et al. 2012; Alvarez-Uria et al. 2018). In view of complete resistance to multiple antibiotic groups, therapy should be focussed on reducing the clinical symptoms and saving the cows that suffer from clinical mastitis caused by MDR *Klebsiella pneumoniae* (Schukken et al. 2012).

The overuse of antibiotics has been suggested to be positively associated with antibiotic resistance in mastitis-causing bacteria. Therefore, their irrational use may not be the ideal solution for mastitis in dairy animals (Saini et al., 2012; Barkema et al. 2015; Kayitsinga et al. 2017). Antibiotic sensitivity test-based selection and prudent use of antibiotics are thus the need of the hour.

**Conclusion**

This study has determined the wide distribution of penicillinase producing *Staphylococcus aureus* and ESBL producing *Escherichia coli* in cows with clinical bovine mastitis. Cephalosporins, tetracyclines, vancomycin and chloramphenicol resistant bacteria were found to be associated with bovine mastitis. Besides, this work has demonstrated the presence of vancomycin resistant enterococci and *Staphylococcus aureus* MDR gram negative rods, MDR Pseudomonas and MDR Acinetobacter, which is an issue of public health concern. Furthermore, the widespread resistance of *Streptococcus uberis* towards cephalosporins, which are commonly used in treating bovine mastitis, has been recorded. MDR transfer from non-pathogens to emerging foodborne and established mastitis pathogens could be a potential problem to the dairy industry and pose public health concerns.

**Author contribution** SPA contributed in conception, design and implementation of the work, reviewed and edited the initial draft of the manuscript. MBK provided technical and laboratory support in sample collection and processing, wrote the initial draft of the manuscript. NVK contributed in statistical analysis.

**Funding** This work did not receive any grant.

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

**Declarations**

**Conflict of interest** None.

**Consent to publish** All authors give consent for publication.

**Ethical approval** Invasive procedure and animal experimentation were not conducted.

**References**

Akhoon ZA, Peer FU, Sofi KA (2012) Study on in vitro sensitivity of bacterial cultures from clinical mastitic milk to few anti-bacterial agents. Indian J Anim Res 46:404–406

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

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

Awandkar SP, Khode NV, Sardar VM, Mendhe MS, Kutkarni 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

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

Barkema HW, van 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

Bernal-Rosa, Y, Osorio-Muñoz K, Torres-García O (2015) *Pseudomonas aeruginosa*: an emerging nosocomial trouble in veterinary. Rev. MVZ Córdoba 20: 4937–4946

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

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 7:479–487
Bradley AJ (2012) Bovine mastitis: an evolving disease. Vet J 164:116–128
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. Vet World 7(6):389–394
Clinical Laboratory Standards Institute (2020) Performance Standards for Antimicrobial Susceptibility Testing M100, 30th edn. Wayne, Accessed 22 June 2021 https://www.mic.org/wp-content/uploads/2021/02/CLSI-2020.pdf
Constable PD, Hinchcliff KW, Don C, Grünberg W (2017) Diseases of the mammary gland. In: Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats, St Louis, Elsevier Ltd. pp. 1904–1996
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. Vet World 10(5):517–520
Gao J, Herman WB, Limei Z, Gang L, Zhaoju D, Jingjie 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
Ghatak S, Singha A, Sen A, Guha C, Ahuja A, Bhattacharjee U, Das S, Pradhan NR, Puro K, Jana C, Dey TK, Prashanthkumar KL, Das A, Shaktunila A, 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
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
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, 2013, Article ID 329121. Accessed 04 Nov 2020 https://doi.org/10.1155/2013/329121
Hendriksen RS, Mevius DJ, Schroeter A, Teale 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. https://doi.org/10.1186/1751-0147-50-28
Hinthong W, Natapop P, Sirijan S, Suphang K, Shutipen B, Nitit S, Wanpen C, Pisinee A, Nitaya I (2017) Detection of antibiotic resistance of *Escherichia coli* from subclinical mastitis cows and water supply in dairy farms in Saraburi Province. PeerJ. https://doi.org/10.7717/peerj.3431
Hirakawa Y, Sasaki H, Kawamoto E, Ishikawa H, Matsumoto T, Aoyama N (2010) Prevalence and analysis of *Pseudomonas aeruginosa* in chinchillas. BMC Vet Res. https://doi.org/10.1186/1746-6148-6-52
Hogeveen H, Huijsk K, Lam T (2011) Economic aspects of mastitis: new developments. NZ Vet J 59:16–23
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. https://doi.org/10.5455/javar.2015.547
Joshi S, Gokhale S (2006) Status of mastitis as an emerging disease in improved and periurban dairy farms in India. Ann NY Acad Sci 1081:74–83. https://doi.org/10.1196/annals.1373.007
Kalmar P, Aasmee 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. https://doi.org/10.1186/1751-0147-53-4
Kateepe DP, Kabugo U, Baluku H, Nyakaruhaka L, Kyobe S, Oke 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. https://doi.org/10.1371/journal.pone.0063413
Kayisinga J, Schewe RL, Contreras GA, Erskine RJ (2017) Anti-microbial 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
Kotwal A, Biswas D, Kakati B, Singh M (2016) ESBL and MBL in ceftaziem resistant *Pseudomonas aeruginosa*: an update from a rural area in Northern India. J Clin Diagn Res. https://doi.org/10.7860/JCDR/2016/18016.7612
Kumar R, Yadav BR, Singh RS (2010) Genetic Determinants of antibiotic resistance in isolates from milk of mastitic crossbred cattle. Curr Microbiol 60:379–386
Lan T, Huimin L, Lu M, Mengru X, Lei D, Mei G, Jiaqi W, Nan Z (2020) Antimicrobial susceptibility, phylogenotypes, and virulence genes of *Escherichia coli* from clinical bovine mastitis in five provinces of China. Food Hydrocolloids 31(1):406–423
Machuca J, Recacha E, Briales A, Diazdebalpa B, Blazquez J, Pascual A (2017) Cellular response to ciprofloxacin in low-level quinolone-resistant *Escherichia coli*. Front Microbiol 8:1370
McMurry L, Levy S (2000) Tetacycline resistance in gram-positive bacteria. In: Gram-positive pathogens. ASM Press, Washington DC pp. 660–677
Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J (2015) Incidence of beta-lactamase producers among *Escherichia coli* isolated from mastitic milk samples. Transbound Emer Dis 63:1080–1085
Mthrowaad M, Kortala S, Birbalsingh R, Huang Q, Ohno K, Kato K, Shimizu M, Yamaguchi T, Malar MS, Gao S, Vaidya S (2019) Detection of emerging antibiotic resistance in bacteria isolated from raw milk samples in Korea. Foodborne Pathog Dis 7:221–224
Nombriga DB, Naushad S, Naqvi SA, Condas LAZ, Saini V, Kastelic JP, Lacy C, De Buck J, Barkema HW (2018) Prevalence and genetic basis of antimicrobial resistance in non-antibiotics *Staphylococci* isolated from Canadian dairy herds. Front Microbiol 9:256. https://doi.org/10.3389/fmicb.2018.00256
Parkala A, Salmikivi L, Bredbacka P, Myllyniemi AL, Koskinen MT (2004) Comparison of tests for detection of beta-lactamase-producing *Staphylococci*. J Clin Microbiol 42:2031–2033
Ranjan R, Gupta MK, Singh S, Kumar S (2010) Current trend of drug sensitivity in bovine mastitis. Vet World 3:17–20
Sadashiv SO, Kaliwal BB (2014) Isolation, characterization and therapeutic options at the turn of the new millennium. Clin Microbiol Infect 13(6):560–578
Minst K, Mörtlbaeuer 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
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
Nobrega DB, Naushad S, Naqvi SA, Condas LAZ, Saini V, Kastelic JP, Lacy C, De Buck J, Barkema HW (2018) Prevalence and genetic basis of antimicrobial resistance in non-antibiotics *Staphylococci* isolated from Canadian dairy herds. Front Microbiol 9:256. https://doi.org/10.3389/fmicb.2018.00256
Pitkala A, Salmikivi L, Bredbacka P, Myllyniemi AL, Koskinen MT (2004) Comparison of tests for detection of beta-lactamase-producing *Staphylococci*. J Clin Microbiol 42:2031–2033
Ranjan R, Gupta MK, Singh S, Kumar S (2010) Current trend of drug sensitivity in bovine mastitis. Vet World 3:17–20
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
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
Saluddin 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
Saluddin M, Mir RA, Khaled Hossain M, Nazir KH, Noreddin A, Mohamed EZ (2020) Molecular detection of multidrug resistant *Staphylococcus aureus* isolated from bovine mastitis milk in Bangladesh. Vet Sci 7:36. https://doi.org/10.3390/vetsci7020036
Salih RRM, Gibreel HH (2019) Gentamicin is the best antibiotic in treatment all types of bovine mastitis caused by bacteria. Agric for J 3(1):46–49
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
Weisblum B (1995) Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother 39:577–585
Yang 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

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.