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**SHORT COMMUNICATION**

# Antibiogram Pattern of Bacterial Isolates Identified from Bovine Clinical Mastitis

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**ABSTRACT**

A total of 65 milk samples from bovine clinical mastitis were collected from villages of Sabarkantha Milk Union and were subjected to bacterial isolation and identification. Out of 74 recovered isolates, *Str. agalactiae* was the most frequent, followed by *E. coli and Str. dysgalactiae*. The *in-vitro* antibiogram revealed that most isolates were sensitive to ceftriaxone/tazobactam, followed by gentamicin, tetracycline and cefoperazone/sulbactam. Ampicillin/cloxacillin combination was found least effective.

**Keywords:** Clinical mastitis, bacterial isolation and identification, antibiogram

Mastitis is one of the major diseases of bovines that seriously affecting the profitability of dairy farmers. Around 70% of the avoidable losses in productivity are reported due to mastitis alone (Sumathi *et al.,* 2008). In India, annual economic losses due to mastitis has been estimated ₹ 7165.51 crores (Bansal and Gupta, 2009). The economic losses associated with clinical mastitis are mainly due to decreased milk production, milk discard during the withholding period after antibiotic usage (together accounting for 25%), veterinary and labour cost, decreased milk quality, culling and, fatalities. Antibiotic usage in dairy animals is by and large attributed to the treatment of mastitis. The emergence of antibiotic resistance is an important issue that has public health implications. Identification of organism and their antibiogram pattern needs to monitor on a regular basis. The present study was conducted to assess the prevalence of bacterial pathogens associated with clinical mastitis in bovines and, to study their antibiogram pattern.

Sixty-five quarter milk samples were collected from bovines with clinical mastitis from different villages of Gujarat. The milk samples were collected aseptically from milch animals by standard milk sampling technique using sterile vials and stored at 4°C until processed (NMC, 2017).

For bacteriology, milk samples were examined by standard protocol (Quinn *et al.*, 2004). For isolation of Gram negative organisms, MacConkey's agar was used. After obtaining growth on MacConkey's agar, Eosine Methylene Blue (EMB) agar was used as the selective media. Mannitol Salt Agar and Edwards medium were used for the isolation of *Staphylococcus* and *Streptococcus,* respectively. For isolation of *Klebsiella* spp., Klebsiella special agar was used. The bacterial isolates were identified based on the cultural, morphological and biochemical characteristics (Edward and Ewing, 1972). Catalase, coagulase, oxidase, latex agglutination test, IMViC (Indole test, methyl-red test, Voges-Proskauer test, citrate utilization test), urease production, glucuronidase, lysine utilization, ortho-Nitrophenyl-β-galactoside (ONPG) test were performed for identification of the bacterial isolates.

*In-vitro* antibiotic sensitivity of the isolates was assessed by disc diffusion method (Bauer *et al.*, 1996) using six antibiotic discs (Hi-Media) namely, gentamicin,

enrofloxacin, ampicillin/cloxacillin, ceftriaxone/ tazobactam, cefoperazone/sulbactam and tetracycline. The antibiotic discs were chosen as per the pattern of use by the veterinarians. Sensitivity was determined on the basis zone of inhibition as per the manufacturer's instructions (Hi- Media).

A total of 74 bacterial isolates were recovered from 65 clinical mastitis milk samples (Table 1). *Str. agalactiae* was the most frequently isolated organism (35.14%), followed by *E. coli* and *Str. dysgalactiae* (20.27% each) and *S. aureus and Klebsiella* spp. (12.16 % each).

Similar prevalence of *Str. agalactiae* has been documented in an earlier report (Jhambh *et al.,* 2012). Earlier, Jeykumar *et al.* (2013) reported isolation of *E. coli* and *Klebsiella* species from bovine clinical mastitis cases. Isolation of *Staphylococcus aureus (*Ghose *et al.,* 2001; Charaya *et al.,* 2014; Lakshmi and Jayavardhanan, 2016) and *Streptococcus dysgalactiae* (Ghose *et al.,* 2001; Charaya *et al.,* 2014; Bhat *et al.,* 2017) have been reported from cases of clinical mastitis by earlier workers.

In our study, *in-vitro* antibiogram pattern revealed high sensitivity towards ceftriaxone/tazobactam (97.3%), followed by gentamicin (87.84%), tetracycline (82.43%) and cefoperazone/sulbactam (79.73%), whereas the organisms were moderately sensitive to enrofloxacin (64.86%). Most of the isolates showed resistance to ampicillin/cloxacillin combination resulting in only a meagre (4.05%) of the isolates being sensitive to the antibiotic combination (Table 1).

*In an earlier study, Staphylococcus* spp., *Streptococcus* spp. and *E. coli* were found sensitive to gentamicin, tetracycline and enrofloxacin (Kaur *et al.*, 2015). In another research study, *S. aureus*, *Str. dysgalactiae* and *E. coli* were reported to be sensitive to ceftriaxone/tazobactam (Bhat *et al*., 2017). Jhambh *et al*. (2012) reported low sensitivity of coliforms to ampicillin/cloxacillin combination. These observations are in accordance with the findings of the present study. The lower sensitivity of most antimicrobials could be attributed to the indiscriminate use of these antibiotics in the field which is an important factor for antibiotic resistance.

With regard to individual isolates, *Str. agalactiae* revealed 80-100 percent sensitivity to ceftriaxone/tazobactam, tetracycline, gentamicin and cefoperazone/sulbactam. A comparatively lower susceptibility was recorded against enrofloxacin (57.69%). Ampicillin/cloxacillin combination was the least effective (7.69%).

*Str. dysgalactiae* isolates exhibited 80-100 percent sensitivity to gentamicin, ceftriaxone/tazobactam and cefoperazone/sulbactam. Relatively lower susceptibility was recorded to tetracycline (60.00%) and enrofloxacin (46.67%). All the *Str. dysgalactiae* isolates showed resistance to ampicillin/cloxacillin combination.

*E. coli* isolates elicited 80-100 percent sensitivity towards ceftriaxone/tazobactam, gentamicin, tetracycline and enrofloxacin. A comparatively lower susceptibility was recorded to cefoperazone/sulbactam (53.33%). Here too, ampicillin/cloxacillin combination was the least effective (6.67%).

**Table 1:** Frequency distribution of mastitis pathogens isolated from mastitis milk samples and their antibiogram pattern

| Organism | Total isolates recovered | No of sensitive isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ceftriaxone/ tazobactam | Gentamicin | Tetracycline | Cefoperazone/ sulbactam | Enrofloxacin | Ampicillin/ cloxacillin |
| *Str. agalactiae* | 26 (35.14) | 26 (100.00) | 23 (88.46) | 24 (92.31) | 23 (88.46) | 15 (57.69) | 2 (7.69) |
| *Str. dysgalactiae* | 15 (20.27) | 14 (93.33) | 14 (93.33) | 9 (60.00) | 13 (86.67) | 7 (46.67) | 0 (0.00) |
| *E.coli* | 15 (20.27) | 14 (93.33) | 12 (80.00) | 12 (80.00) | 8 (53.33) | 12 (80.00) | 1 (6.67) |
| *S. aureus* | 9 (12.16) | 9 (100.00) | 8 (88.89) | 9 (100.00) | 9 (100.00) | 7 (77.78) | 0 (0.00) |
| *Klebsiella spp.* | 9 (12.16) | 9 (100.00) | 8 (88.89) | 7 (77.78) | 6 (66.67) | 7 (77.78) | 0 (0.00) |
| **Total** | **74** | **72 (97.30)** | **65 (87.84)** | **61 (82.43)** | **59 (79.73)** | **48 (64.86)** | **3 (4.05)** |

*percentage in parenthesis.

Antibiogram pattern for *S. aureus* revealed 70-100 percent sensitivity to ceftriaxone/tazobactam, tetracycline, cefoperazone/sulbactam, gentamicin and enrofloxacin. None of the *S. aureus* isolates exhibited sensitivity to ampicillin/cloxacillin combination.

Isolates of *Klebsiella spp.* elicited 70-100 percent sensitivity towards ceftriaxone/tazobactam, gentamicin, enrofloxacin and tetracycline. Relatively lower sensitivity was recorded to cefoperazone/sulbactam (66.67%). All the isolates were resistant to ampicillin/cloxacillin combination.

## CONCLUSION

Bacteriological isolation, biochemical characterization of mastitis causing organisms and subsequently determining their antibiotic susceptibility is important in the therapeutic management of bovine clinical mastitis.

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## COMPETING INTEREST

The authors declare that they have no competing interests.

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