Journal of Animal Research: v.10 n.6, p. 1037-1042. December 2020

DOI: 10.30954/2277-940X.06.2020.24

Seasonal Prevalence and Antibiogram Studies of Bovine Mastitis in Southern Haryana

Jinu Manoj[1*] and Manoj Kumar Singh[2]

[1]College Central Laboratory, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, INDIA

[2]Department of Livestock Production and Management, College of Veterinary and Animal Sciences, SVPUAT, Meerut, U.P., INDIA

**Corresponding author: J Manoj; E-mail: drjinumanoj@gmail.com*

Received: 17 Sept., 2020 **Revised:** 26 Nov., 2020 **Accepted:** 10 Dec., 2020

ABSTRACT

Mastitis in bovines causes huge economical loss to the dairy industry. It affects the public health due to the transmission of pathogenic bacteria and the toxins produced by them through the food chain. The timely diagnosis of subclinical mastitis (SCM) and its proper treatment based on antibiogram have significant impact on reducing the economical loss to the dairy farmers. Therefore the present study was conducted to find out the prevalence and antibiogram pattern of the mastitis causing bacteria in Southern Haryana. A total prevalence rate of 65.79% was observed for subclinical mastitis in Southern Haryana by the screening test. The highest prevalence was observed in winter (59.49%), followed by rainy season (69.61%) and cattle were found as more susceptible to SCM when compared to buffaloes. The major Gram negative pathogens isolated were belonged to *E. coli*, followed by *Klebsiella* species and *Pseudomonas* species, the common organisms responsible for environmental mastitis. The antibiogram patterns of the isolates were also studied and the maximum sensitive antibiotic against mastitis was found as Chloramphenicol, followed by Enrofloxacin, Amikacin and Ampicillin. The most resistant antibiotics in the study area was found as Ceftizoxime followed by Amoxicillin as these antibiotics were frequently and indiscriminately used for the treatment of mastitis in the study area without subjecting to *in vitro* antibiotic sensitive test. This emphasis the need of awareness among dairy farmers regarding the hygienic managemental practices to be followed at farm level as well as necessity of conducting antibiotic sensitivity test for the treatment.

HIGHLIGHTS

- A total prevalence of mastitis observed was 65.79%.
- The most resistant antibiotics were Ceftizoxime and Amoxicillin.
- The most sensitive antibiotics were Chloramphenicol and Enrofloxacin.

Keywords*:* Antibiogram, Haryana, Mastitis, Prevalence, Seasonal

Mastitis the potentially fatal udder tissue infection is the most common disease in dairy animals. It usually occurs due to the inflammatory response to bacterial invasion of the teat canal. It can also occur as a result of chemical, mechanical or thermal injury to the udder (Akhoon *et al*., 2015). It affects the public health due to the transmission of pathogenic bacteria and their toxins through the food chain (Argaw, 2016; Shaheen *et al*., 2016; Hameed *et al*., 2007). The prevalence of subclinical mastitis (SCM) is more prevalent than clinical mastitis (Sharma *et al*., 2012). Meta analysis revealed 29-45% increased prevalence of subclinical mastitis during 2011 to 2016 (Krishnamoorthy *et al*., 2017). The subclinical mastitis lacks visible inflammatory changes initially and for the

How to cite this article: Manoj, J. and Singh, M.K. (2020). Seasonal Prevalence and Antibiogram Studies of Bovine Mastitis in Southern Haryana. *J. Anim. Res.,* **10**(6): 1037-1042.

Source of Support: None; **Conflict of Interest:** None

detection of it, the laboratory examination is necessary. Mastitis in bovines causes huge economical loss to the livestock industry globally and affects international trade of milk and milk products (Shaheen *et al*., 2016). The economical loss due to mastitis in Indian conditions is also available (Devi and Dutta, 2018). The sub clinically affected animals can act as continuous source of infection to other animals also (Dasohari *et al*., 2017). If untreated, SCM leads eventually to clinical mastitis and will be difficult to cure completely. The indiscriminate use of antibiotics for the treatment of mastitis without checking the *in vitro* sensitivity pattern leads to treatment failure as well as development of antibiotic resistance in addition to the financial burden (Shaheen *et al*., 2016). Antibiotic resistance among mastitogens is well reported in India (Tufani *et al*., 2012; Charaya *et al*., 2013; Akhoon *et al*., 2015; Chandrasekharan *et al*., 2015; Das *et al*., 2017). The monitoring of antibiotic resistance in livestock is recommended by OIE and WHO recommends a rational and judicious use of antibiotics in public domain (Dougnon *et al*., 2020). The antibiotic resistance a global concern can be minimised by the discriminate as well as proper monitoring of its use in livestock sector. The timely diagnosis of subclinical mastitis and its proper treatment based on antibiogram have significant impact on reducing the economical loss to the dairy farmers (Argaw, 2016). Therefore, the present study was conducted to find out the prevalence and antibiogram pattern of the mastitis causing bacteria in Southern Haryana.

MATERIALS AND METHODS

Milk Samples

This one year study was conducted from June 2018 to May 2019. A total of 6738 milk samples brought to the Disease Investigation Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Mahendragarh, Haryana were examined for subclinical mastitis. Out of these, 5196 and 1542 samples were from buffaloes and cows, respectively. The study period was divided into four season's *viz*., summer, winter, spring/autumn and rainy (Table 1).

Screening test

The milk samples were screened for subclinical mastitis by modified whiteside test using 4% sodium hydroxide solution. The nature of coagulation and viscosity formed after adding the reagent with equal amount of milk was noted for diagnosing the subclinical mastitis and the severity of the infection.

Phenotypical characterization

A total of 214 milk samples (107 samples each from both the species of study) which were found as positive for subclinical mastitis by the screening test were further subjected to microbiological analysis (Quinn *et al*., 2011). The isolation and identification of Gram negative organisms were attempted from these samples. Briefly, the positive milk samples were streaked onto MacConkey Lactose agar and incubated overnight at 37°C aerobically. The bacterial colonies developed after the incubation was identified on the basis of morphological and phenotypical features such as colony size, shape, colour development and biochemical characteristics. Gram negative bacteria were identified by diverse biochemical reactions such as IMViC test, urease test, H_2S production and fermentation of sugars. Gram staining of the pure colonies was also performed using Gram's stain kit K001L (HiMedia Lab. Pvt. Ltd., Mumbai).

Antibiotic sensitivity studies

The antibiogram patterns of the isolates were also studied as per the standard protocol (Kirby-Bauer's disc diffusion technique) in brain heart infusion agar using guidelines of CLSI (Bauer *et al*., 1966). A total of 12 antibiotic discs were used (Table 2) to study the susceptibility pattern of the Gram negative mastitogens. The sensitivity and the resistance patterns of the organism were recorded by comparing the diameter of growth inhibition zone developed with the zone diameter interpretation chart provided by the manufacturer (HiMedia Lab. Pvt. Ltd., Mumbai) of the antibiotic discs.

RESULTS AND DISCUSSION

Prevalence rate

In the present study a total prevalence rate of 65.79% was observed for subclinical mastitis in Southern

Haryana by the screening test. Among these, 3271 and 1162 samples were found as positive from buffaloes and cattle, respectively with 62.95% and 75.35% species-wise prevalence. The cows are more prevalent for mastitis than buffaloes (Sharma *et al*., 2012; Devi and Dutta, 2018). The season wise prevalence of SCM is depicted in Table 1 with highest prevalence observed in winter (59.49%), followed by rainy season (69.61%).

Table 1: Milk samples examined (Season-wise) for mastitis during 2018-2019

Season*	Species	No. of samples examined	No. of samples positive	Prevalence (%)
	Buffalo	1525	1004	65.83
Rainy	Cattle	670	524	78.20
	Total	2195	1528	69.61
	Buffalo	1516	868	57.25
Spring/ Autumn	Cattle	291	207	71.13
	Total	**1807**	**1075**	**59.49**
	Buffalo	1606	1131	70.42
Winter	Cattle	254	198	77.95
	Total	**1860**	**1329**	**71.45**
	Buffalo	549	268	48.81
Summer	Cattle	327	233	71.25
	Total	**876**	**501**	**57.19**
	Buffalo	5196	3271	62.95
Total	Cattle	1542	1162	75.35
	Total	**6738**	**4433**	65.79

*Rainy-July, August, September; Spring/Autumn-October, November, March; Winter-December, January, February; Summer-April, May, June

The prevalence of subclinical mastitis reported varied widely with respect to geography, agro climatic conditions and management practices of different area. Palanivel *et al*. (2008) reported highest occurrence of mastitis in summer from Tamilnadu, while Kurjogi and Kaliwal (2014) from Karnataka reported highest prevalence during monsoon season followed by winter. Tufani *et al.* (2012) found highest mastitis prevalence in spring season followed by winter. Prevalence rate of mastitis varies from place to place, herd to herd and country to country (Devi and Dutta, 2018). Various researchers across the State reported different prevalence rates of SCM in their studies. Charaya *et al*. (2018) and Pankaj *et al*. (2012) reported 33.76% and 64.21% prevalence of subclinical mastitis in buffaloes and cows respectively from Hisar, Haryana. Jhambh *et al*. (2017) found 31.79% prevalence of SCM in buffaloes from an organized farm located in Haryana. Bhanot *et al*. (2012) reported a prevalence rate of 85.30% in cows and 78.1% in buffaloes, respectively in Eastern Haryana. Kumar and Sharma (2002) reported 59.86% and 66.27% prevalence of SCM in cows and buffaloes, respectively from Hisar, Haryana. The prevalence rate of SCM from various parts of India also reported. Swami *et al*. (2017) reported a prevalence rate of 35% in cows and 28.33% in buffaloes, respectively from Latore, Maharashtra. Gogoi *et al*. (2017) reported a prevalence rate of 19.56% in cows from Assam, while Javia *et al*. (2018) reported 34.29% of SCM from Junagarh, Gujarat. Bhaghel *et al.* (2018) from Mhow, Madhya Pradesh, Sharma *et al*. (2007) from Chatissgarh and Dasohar *et al.* (2017) from Hyderabad, Telangana recorded 43.6%, 66% and 66.18% positivity of SCM in their studies, respectively. Kurjogi and Kaliwal (2014) recorded 46% prevalence from Karnataka. Kumari *et al*. (2018) reviewed about subclinical mastitis of cattle with emphasis on prevalence, risk factors and economical losses in various States of India.

In this study, a total of 67 Gram negative bacterial isolates were isolated from the mastitic milk samples of buffaloes, while only 43 Gram negative bacterial isolates were obtained from cows. The major pathogens isolated from buffaloes belonged to *E. coli* (50.74%), followed by *Klebsiella* sp. (40.29%) and *Pseudomonas* sp. (5.97%). One isolate of *Proteus* sp. and *Salmonella* sp. was also found from mastitic buffaloes. In case of cows, 19 isolates were of *E. coli* (57.57%), while *Klebsiella* sp. was of 33.33% and *Pseudomonas* sp. was of 9.09%. The major pathogens for environmental mastitis are *E. coli*, *Klebsiella* and *Proteus* (Shaheen *et al*., 2016). The presence of pathogens from the infection depends on several risk factors such as quality of managemental practices followed at farm and milking level (Argaw, 2016). Animal which are frequently exposed to constant soiling of udder due to improper housing and hygienic conditions are more likely to have mastitis caused by environmental pathogens like *E. coli* (Sharma *et al*., 2007; Akhoon *et al*., 2015).

Antibiotic sensitivity studies

The *in vitro* antibiotic sensitivity test data obtained were expressed in percentage of sensitivity and resistance against Gram negative bacterial isolates (Fig. 1).

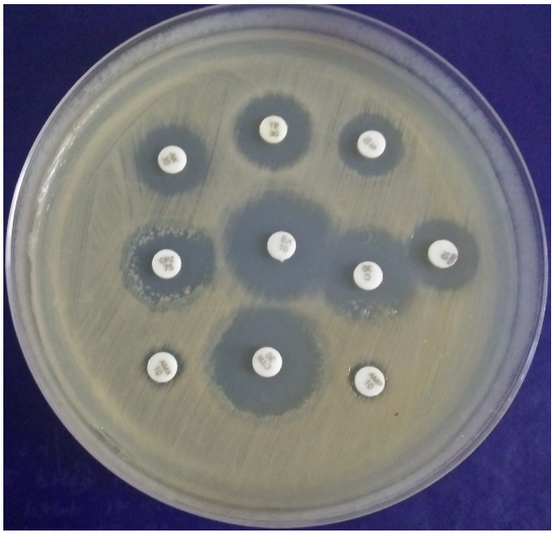

Fig. 1: Antibiotic Sensitivity Test

In the present investigation, the maximum sensitive antibiotic against subclinical mastitis in buffaloes from Southern Haryana was found as Chloramphenicol (65.63%) followed by Enrofloxacin (56.25%), Amikacin (47.10%) and Ampicillin (36.73%) (Table 2). The most resistant antibiotics against the mastitogens in the study area were found as Ceftizoxime (66.37%), followed by Amoxicillin (58.10%) and Ceftriaxone (39.02%). The antibiotics, Cefoperazone (47.35%), Amoxyclav (52.23%), Oxytetracycline (44.93%), Gentamicin (42.52%) and Streptomycin (38.51%) were found as intermediate in susceptibility in majority of the mastitic cases from buffaloes. The studies reported from various States of India gives an indication about the antibiotic resistance pattern of subclinical mastistis across the country. Akhoon *et al*. (2015) found cent percentage sensitivity to Gentamicin, while Ampicillin and Amoxicillin showed complete resistance in their studies from Kashmir. Sharma *et al*. (2007) also reported highest sensitivity to Gentamicin by *E. coli* isolates. Chandrasekaran *et al.* (2015) reported more sensitivity to Enrofloxacin and highest resistance to penicillin followed by Amoxicillin against *E. coli* isolates from Tamil Nadu. Bhanot *et al*. (2012) observed Enrofloxacin as most sensitive drug at eastern Haryana, while Bhat *et al*. (2017) from Jammu reported Enrofloxacin and Gentamicin as most sensitive drug against *E. coli* and Penicillin as the least effective antibiotic.

Table 2: *In vitro* antibiotic sensitivity of Gram negative isolates from cases of mastitis in buffaloes

Sl. No.	Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
1	Chloramphenicol	65.63	18.75	15.62
2	Enrofloxacin	56.25	22.87	21.88
3	Amikacin	47.10	36.33	16.57
4	Ampicillin	36.73	42.04	21.23
5	Cefoperazone	30.13	47.35	22.52
6	Amoxyclav	29.40	52.23	18.37
7	Oxytetracycline	32.79	44.93	22.28
8	Gentamicin	28.93	42.52	28.55
9	Streptomycin	29.17	38.51	32.32
10	Ceftriaxone	21.16	33.82	39.02
11	Amoxicillin	13.28	28.62	58.10
12	Ceftizoxime	15.46	18.17	66.37

Table 3: *In vitro* antibiotic sensitivity of Gram negative isolates from cases of mastitis in cows

Sl. No.	Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
1	Chloramphenicol	71.62	14.97	14.01
2	Enrofloxacin	62.61	17.77	19.62
3	Amikacin	51.40	28.97	19.63
4	Ampicillin	42.05	31.77	26.18
5	Cefoperazone	34.57	51.40	14.03
6	Amoxyclav	33.64	45.79	20.57
7	Oxytetracycline	30.08	37.99	31.93
8	Gentamicin	29.90	32.05	38.05
9	Streptomycin	27.10	26.44	46.46
10	Ceftriaxone	26.16	28.05	45.79
11	Amoxicillin	25.33	30.84	43.93
12	Ceftizoxime	23.36	28.98	47.66

Almost similar antibiotic sensitivity trend was observed in mastitits from cattle too (Table 3), eventhough the percentage of sensitivity was a little higher among them. In case of cattle, the most sensitivity was found for Chloramphenicol (71.62%) followed by Enrofloxacin (62.61%), Amikacin (51.40%) and Ampicillin (42.05%)

with highest resistance for Ceftizoxime (47.66%) followed by Streptomycin (46.46%), Ceftriaxone (45.79%) and Amoxicillin (43.93%). In this study, the maximum sensitivity was observed for Amphenicol group of antibiotics, followed by Fluoroquinolones. It was further observed from the history of cases that the most resistant antibiotics found in this study were in field use, frequently and indiscriminately, since a long time for the treatment of mastitis in the study area, without subjecting to *in vitro* antibiotic sensitive test. Several researchers reported similar problems in case of bovine mastitis across the country, which leads to antibiotic resistance eventually (Awandkar *et al*., 2013; Akhoon *et al*., 2015; Shaheen *et al*., 2016; Bhat *et al*., 2017).

CONCLUSION

Mastitis become a concern for dairy farmers, dairy processors, consumers and society due to its high incidences, production losses, milk quality, animal welfare, antimicrobial resistance and residue issues. The presence of environmental pathogens in this study strongly recommends the need for hygienic management measures to be followed at farm level to prevent environmental mastitis. The study emphasis the needs of investigation of mastitis epidemiology as well as the awareness among dairy farmers regarding the necessity of conducting antibiotic sensitivity test also. This in turn, will increase the income of dairy farmer through reduced treatment cost, ensuring better livestock welfare and increased milk production with premium benefits in the dairy industry.

REFERENCES

Akhoon, Z.A., Peer, F.U. and Nazir, A. 2015. Epidemiology of mastitis in Kashmir valley. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*., **36**: 43-45.

Argaw, A. 2016. Review on epidemiology of clinical and subclinical mastitis on dairy cows. *Food Sci. Qual. Manag.,* **52**: 56-65

Awandkar, S.P., Bhikane, A.U. and Kulkarni, M.B. 2013. Antibiotic resistance trends in clinical bovine mastitis. *Biolife.,* **1**: 139-143.

Baghel, A., Chhabra, D., Sharda, R., Shukla, S., Audarya, S., Sikrodia, R. and Gangil, R. 2018. Isolation of *Staphylococcus* from bovine mastitis and their antibiotic sensitivity pattern. *Indian J. Vet. Sci. Biotechnol*., **13**: 49-52.

Bauer, A.W., Kieby, W.M.M., Shrenis, J.C. and Turck, M. 1966. Antibiotic susceptibity testing by a standardized single disc diffusion method. *American J. Clin. Pathol*., **45**: 453-496.

Bhanot, V., Chaudhri, S.S., Bisla, R.S. and Singh, H. 2012. Retrospective study on prevalence and antibiogram of mastitis in cows and buffaloes of eastern Haryana. *Indian J. Anim. Res*., **46**:160-163.

Bhat, A.M., Soodan, J.S., Singh, R., Dhobi, I.A., Hussain, T., Dar, M.Y. and Mir, M. 2017. Incidence of bovine clinical mastitis in Jammu region and antibiogram of isolated pathogens. *Vet. World.,* **10**: 984-989.

Chandrasekaran, D., Nambi, A.P., Thirunavukkarasu, P.S., Venkatesan, P., Tirumurugaan, K.G. and Vairamuthu, S. 2015. Incidence of resistant mastitis in dairy cows in Tamil Nadu, India. *J. Appl. Nat. Sci*., **7**: 304-308

Charaya, G., Sharma, A., Singh, M., Tiwari, S., Pankaj and Kumar, A. 2013. Subclinical mastitis at an organised farm: prevalence, etiology and antibiogram. *Haryana Vet.,* **52**: 30-32.

Das, A., Guha, C., Biswas, U., Jana, P.S., Chatterjee, A. and Samanta, I. 2017. Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal, *Vet. World*., **10**: 517-520.

Dasohari, A., Somasani, A., Nagaraj, P and Reddy G. A. 2017. Epidemiological studies of subclinical mastitis in cows in and around Hyderabad. *Pharma Innov. J*., **6**: 975-979.

Devi, M. and Dutta, J.B. 2018. Incidence of bovine subclinical mastitis in organized and unorganized farms based on somatic cell count. *Int. J. Chem. Stud*., **6**: 1399-1403.

Dougnon, V., Chabi, Y., Koudokpon, H., Agbankpe, J., Sefounon, R., Alle, D., Bankole, H. and Baba-Moussa, L. 2020. Prescription of antibiotics as a source of emerging antibiotic resistance: Knowledge, attitudes, and practices of medical staff in the Dassa-Glazoue and Savalou-Bante's health zones (Benin, West Africa). *Int. J. One Health*., **6**: 34-40.

Gogoi, S.M., Tamuly, U.R., Khuman, L.S., Saikia, G.K. and Bordoloi, G. 2017. Prevalence of sub clinical mastitis in areas around Lakhimpur town of Assam. *Int. J. Agri. Sci. Res.,* **7**: 501-508.

Hameed, K.G.A., Sender, G. and Korwin-Kossakowaska, A. 2007. Public health hazard due to mastitis. *Anim. Sci. Pap. Report*., **25**: 73-85.

Javia, B., Purohit, J., Mathapati, B., Barad, D., Savsani, H., Ghodasara, S., Kalariya, V., Patel, U. and Nimavat, V. 2018. Molecular detection and antimicrobial resistance pattern of *Staphylococci* isolated from clinical and subclinical bovine mastitis. *Indian J. Vet. Sci. Biotechnol.,* **14**: 13-16.

Jhambh, R., Dimri, U., Gopala Krishnan, A., Singh, M. and Chhabra, R. 2017. Prevalence and risk factors of subclinical

mastitis in buffaloes at an organized dairy farm in western Haryana. *Haryana Vet*., **56**: 189-193.

Krishnamoorthy, P., Suresh, K.P., Saha, S., Govindaraj, G., Shome, B.R. and Roy, P. 2017. Meta analysis of prevalence of subclinical and clinical mastitis, major mastitis pathogens in dairy cattle in India. *Int. J. Current Microbiol. Appl. Sci.,* **6**: 1214-1234.

Kumar, R. and Sharma, A. 2002. Prevalence, etiology and antibiogram of mastitis in cows and buffaloes in Hisar, Haryana. *Indian J. Anim. Sci*. **72**: 361-363.

Kumari, T., Bhakat, C. and Choudhary, R.K. 2018. A review on sub clinical mastitis in dairy cattle, *Int. J. Pure App. Biosci*., **6**: 1291-1299.

Kurjogi, M.M. and Kaliwal, B.B. 2014. Epidemiology of bovine mastitis in cows of Dharwad district. *Int. Schol. Res. Notice*., **2014**: 1-9. Article ID 968076.

Pankaj, Sharma, A., Chhabra, R. and Sindhu, N. 2012. Prevalence of sub clinical mastitis in cows: its etiology and antibiogram. *Indian J. Anim. Res*., **46**: 348-353.

Quinn, P.J., Markey, B.K., Leonard, F.C., Fitz Patrick, E.S., Fanning, S. and Hartigan, P.J. 2011. Veterinary Microbiology and Microbial Diseases, 2nd Ed., Wiley Blackwell, John Wiley & Sons Ltd, West Sussex, UK, pp. 115-292.

Shaheen, M., Tantary, H.A. and Nabi, S.U. 2016. A treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy. *J. Adv. Dairy Res.,* **4**: 150.

Sharma, N., Maiti, S.K. and Sharma K.K. 2007. Prevalence, etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). *Int. J. Dairy Sci*., **2**: 145-151.

Sharma, N., Rho, G.J., Hong, Y.H., Kang, T.Y., Lee, H.K., Hur, T. and Jeong, D.K. 2012. Bovine mastitis: An Asian perspective. *Asian J. Anim. Vet. Adv*., **7**: 454-476.

Sharma, A., Chhabra, R., Singh, M. and Charaya, G. 2018. Prevalence, etiology and antibiogram of bacterial isolates recovered from mastitis of buffaloes. *Buffalo Bulletin*., **37**: 313-320.

Swami, S.V., Patil, R.A. and Gadekar, S.D. 2017. Studies on prevalence of subclinical mastitis in dairy animals. *J. Entomol. Zool. Stud.,* **5**: 1297-1300.

Tufani, N.A., Makhdoomi, D. M. and Hafiz, A. 2012. Epidemiology and therapeutic management of bovine mastitis. *Indian J. Anim. Res*., **46**: 148-151.