Microbiology Research Journal International

Volume 34, Issue 4, Page 1-12, 2024; Article no.MRJI.113597
ISSN: 2456-7043, NLM ID: 101726596
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

A Temporal Study on Incidence of Bovine Mastitis in Haryana, India

Jinu Manoj [a++*], Rajesh Chhabra [a], Mahavir Singh [a], and Manoj Kumar Singh [b]

[a] *College Central Laboratory, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.*
[b] *College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2024/v34i41437

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113597

Original Research Article

Received: 24/01/2024
Accepted: 27/02/2024
Published: 12/04/2024

ABSTRACT

Mastitis affects dairy animal's productivity and causes financial losses for dairy farmers in India and across the world. In this study, a total of 52,494 quarter milk samples from 14,381 bovines were screened for the primary microorganisms causing mastitis in Hisar and adjoining districts of Haryana and their antibiotic sensitivity patterns were analyzed. The cultural positivity from subclinical form of mastitis was observed as 86.32% and 87.73% from cows and buffaloes, respectively while that from clinical mastitis was 87.36% and 87.57%. The major Gram positive bacterial pathogens associated with mastitis in the entire study period were found as *Staphylococcus* species with an average incidence rate of 45.53% and 44.1% from cows and buffaloes, respectively. The *Streptococcus* species were found to be 33.76% and 29.94% of total isolates. *Escherichia coli* were the most predominant Gram negative bacteria isolated (17.37% and 13.85%), thereafter *Klebsiella* spp. (5.54% and 5.19%) from both cows and buffaloes. A significant proportion of clinical cases of

[++] *Disease Investigation Officer;*
**Corresponding author: E-mail: drjinumanoj@gmail.com;*

mastitis were chronic in nature from both the species of bovines. The incidence of mastitis with respect to different lactation number and lactation months was found as significant. The highest incidences of mastitis was observed in the first lactation among buffaloes (20.69%), while the maximum incidences were observed in second and third lactation in cows with 16.59% and 16.99%, respectively. However, in both the species maximum occurrence of mastitis was observed during the first lactational month. The antibiotic sensitivity patterns of most of the isolates had shown higher sensitivity towards enrofloxacin and gentamicin, while the penicillin had shown least sensitivity. The knowledge regarding the mastitis causing pathogens and their sensitivity pattern in Hisar and adjoining districts of Haryana enables the veterinarians to adopt for the proper treatment protocols and dairy farmers to assure optimal health, welfare and productivity of bovines in the State, in turn reducing antimicrobial resistance.

Keywords: Antimicrobial resistance; bovines; Haryana; mastitis; staphylococcus, E. coli.

1. INTRODUCTION

Mastitis is the most common disease that affects dairy animal’s productivity. Dairy farmers in low and middle income countries, including India, suffer financial losses as a result of mastitis in terms of decreased production as well as treatment and prevention costs [1,2]. The inflammatory response to bacterial invasion of the teat canal and udder parenchyma results in mastitis [3]. The spread of harmful microorganisms and their toxins through the milk and dairy products has an impact on public health [4,5]. Intramammary infections can occur in various forms like asymptomatic, subclinical or clinical form. Acute or chronic infections are associated with clinical symptoms [6].

Mastitis is a very challenging disease due to the involvement of diverse groups of pathogens. The etiological agents most commonly involved in bovine mastitis are either of contagious or environmental pathogens. Mastitis management and treatment are greatly influenced by the interactions between the pathogen, animals, farm environment and management factors [7]. In addition to depending on the animal's stage of production and the disease's clinical manifestation, the probability of isolating the causative agents can change across time and space [8]. The administration of antibiotics either by parenteral or intramammary routes during the treatment of mastitis in dairy animals has an association to the emergence of microorganisms that are resistant to antibiotics and subsequent treatment failure.

Antimicrobial resistance is a global issue that affects both human and animal health. Concerns about the emergence and spread of antibiotic resistance have prompted action in the field of animal health [9]. Antibiotic resistance among mastitis causing pathogens is reported from India [3,10-13]. This is particularly crucial when antibiotics are administered to dairy animals as a form of dry cow therapy without identifying the bacteria responsible for the disease and its antimicrobial sensitivity profile. The OIE advises monitoring antibiotic resistance in animals while WHO urges prudent and sensible use of antibiotics in the community as a whole [14].

Mastitis-affected bovines could transmit the infection to other susceptible animals in the herd [15]. Antimicrobial resistance can be minimized by using antibiotics prudently in livestock production. Early detection of mastitis, effective management and antibiogram based treatment has a substantial impact on minimizing the financial loss to dairy farmers [4]. In this study, we had analyzed the data from our laboratory and focused on the major pathogens causing mastitis in Hisar, Haryana and adjoining districts and their sensitivity pattern for the antibiotics.

2. MATERIALS AND METHODS

2.1 Samples

This retrospective study analyzed the data from 1 July 2019 to 31 June 2023. Milk samples received in aseptic conditions at the College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana for the bacteriological examination were used in this study. These samples were routinely submitted to the laboratory for the cultural examination and antibiotic sensitivity testing either by the dairy farmers or veterinarians. The details of each case were recorded from the owner of the animal and it included species, breed, age/lactation number, milk yield, symptoms and its duration *etc* along with clinical findings. The clinical case of mastitis is again categorized into four groups according to the nature of clinical signs *viz*., peracute, acute, sub acute or chronic [6].

2.2 Isolation of the Bacteria

The milk samples were subjected to bacteriological analysis as per the standard method [16]. The bacterial isolation of both Gram positive as well as Gram negative organisms were performed separately. Briefly, a representative sample (10µl) of milk sample from each quarter was inoculated onto blood agar containing 5% defibrinated sheep blood (SBA) and Mac Conkey's Lactose agar (MLA) plates, simultaneously after proper mixing and under aseptic conditions. These plates were incubated aerobically at 37^0C. The plates were checked for bacterial growth after 16-18 hours of incubation. If no growth were observed, then the plates were reincubated upto 48 hours. The colonies developed on the SBA and MLA was identified presumptively, based on their morphological and phenotypical features such as colony size and shape, haemolytic pattern, colour development, biochemical tests and Gram staining.

2.3 *In vitro* Antimicrobials Sensitivity Testing

The bacterial isolates obtained were tested for their antibiotic susceptibility pattern using the Kirby-Bauer disc diffusion method according to CLSI guidelines [17]. A total of 16 different antibiotics from six classes were used, which include amoxicillin (10µg), ampicillin (10µg), amikacin (30µg), chloramphenicol (30µg),cloxacillin (30µg), cefoperazone (75g), ceftriaxone (30µg), enrofloxacin (10µg), gentamicin (10µg), levofloxacin (5µg), moxifloxacin (5µg), neomycin (30µg), oxytetracycline (30µg), penicillin (10units) and streptomycin (10µg). The isolates were classified as resistant, intermediate or sensitive towards the tested antibiotics based on the zone of inhibition developed as per manufacturer's quality control instructions (HiMedia laboratories Pvt. Ltd, Mumbai, India).

2.4 Statistical Analysis

Statistical analysis was carried out using chi-squared test and odds ratio at 95% confidence interval (IBM SPSS Statistics version 21, New York). The differences were considered as statistically significant at $P \leq 0.05$ between parameters from cattle and buffaloes.

3. RESULTS AND DISCUSSION

3.1 Bacteriological Examination

A total of 52,494 quarter milk samples from 14,381 bovines were analyzed by cultural method in this study. These samples were obtained from 3,990 cows and 10,391 buffaloes of Haryana State and adjoining States of India. Among these, a total of 7,202 samples were from subclinical cases of mastitis, while 7,179 samples were from clinical cases. The bacteriological examination of samples revealed that, on an average 87.36% and 86.32% of quarters from cows were culturally positive for different bacterial pathogens from clinical and subclinical form of mastitis, respectively. Similarly, 87.57% and 87.73% of quarters from clinical and subclinical mastitic cases of buffaloes were also found as culturally positive. The year wise details of samples processed for milk culture are given in Table 1. The second and fourth year of study period had shown a significant difference with p value less than 0.05 from both clinical and subclinical cases of cows and buffaloes, except the subclinical cases of cows which showed significance during second and third year.

A meta-analysis study reported a pooled estimate of clinical mastitis as 16.08% in crossbred cows [18], while another reported a pooled prevalence of 41% and 27% for subclinical and clinical mastitis, respectively from India [19]. However, a pooled prevalence of 42% and 15% was reported in the World for the subclinical and clinical mastitis, respectively [20]. The bacteriological analysis of samples received at our lab was given an average cultural positivity of 87.23%. This is in accordance with the other reports from various States of India. The percentages of bovines with subclinical mastitis in India were ranged from 9.88 to 86.87% [21]. The overall prevalence of mastitis from eastern Haryana over a period of six years was found as 81.7% [22], while a prevalence rate of 65.79% was reported from Southern Haryana [13]. Subclinical mastitis in the States of Punjab, Madhya Pradesh and Assam were also reported with 19.2 to 83%, 41.66% and 93.33%, respectively [23,24,25]. A total of 85,677 isolates were obtained from infected quarters of bovines in this study (Table 2). Among these, 74.73% of isolates were from buffaloes, while of 25.27% were from cows. The isolates from clinical cases of mastitis were 48.34% and 51.66% were from subclinical samples. The total annual proportion of isolates obtained from clinical and subclinical mastitis samples were found as significantly different (P <0.05) with high chi-square value.

Table 1. The milk samples processed for bacteriological examination by cultural method

Year	Buffaloes											
	Clinical cases (n=4667)						Subclinical cases (n=5724)					
	Quarters examined	Quarters culturally positive	Percentage	Odds ratio	95 % CI	P value	Quarters examined	Quarters culturally positive	Percentage	Odds ratio	95 % CI	P value
2019-20	3528	3040	86.17				5808	5026	86.54			
2020-21	3893	3423	87.93	0.86	0.747-0.979	0.0241	4551	4023	88.39	0.84	0.749-0.949	0.0047
2021-22	4826	4166	86.32	0.99	0.870-1.119	0.8376	5392	4670	86.61	0.99	0.891- 1.108	0.9087
2022-23	4814	4326	89.86	0.70	0.615-0.803	< 0.0001	5276	4716	89.39	0.76	0.679- 0.857	< 0.0001
Total	**17061**	**14955**	**87.57**				**21027**	**18435**	**87.73**			

Year	Cows											
	Clinical cases (n=2512)						Subclinical cases (n=1478)					
	Quarters examined	Quarters culturally positive	Percentage	Odds ratio	95 % CI	*P* value	Quarters examined	Quarters culturally positive	Percentage	Odds ratio	95 % CI	*P value*
2019-20	1928	1655	85.84				1627	1406	86.42			
2020-21	1907	1712	89.77	0.69	0.568-0.839	0.0002	1087	995	91.54	0.59	0.455-0.76	< 0.0001
2021-22	2544	2147	84.39	1.12	0.945-1.325	0.1799	1279	1039	81.24	1.47	1.204-1.794	0.0002
2022-23	2612	2336	89.43	0.72	0.599-0.856	0.0003	1422	1224	86.08	1.03	0.837-1.265	0.785
Total	**8991**	**7850**	**87.36**				**5415**	**4664**	**86.32**			

Table 2. Annual proportion of bacterial isolates obtained from the milk samples of the study Mastitis associated pathogens

Year	Cows		Buffaloes		Total	χ2	*P* value
	Clinical cases	Subclinical cases	Clinical cases	Subclinical]cases			
2019-20	2863	2531	5707	9375	20476	231.3715	0.00001
2020-21	3321	1945	7479	8675	21420		
2021-22	3957	2040	8235	9738	23970		
2022-23	3196	1797	6655	8163	19811		
Total	13337	8313	28076	35951	85677		

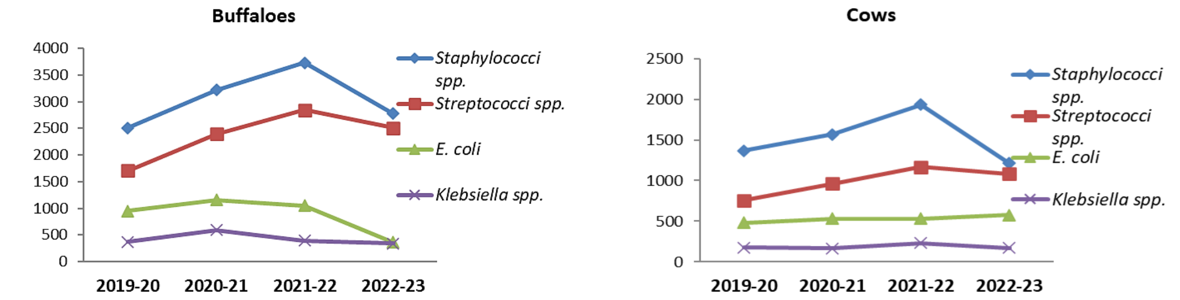

Fig. 1. Major pathogens isolated from bovine clinical mastitis cases

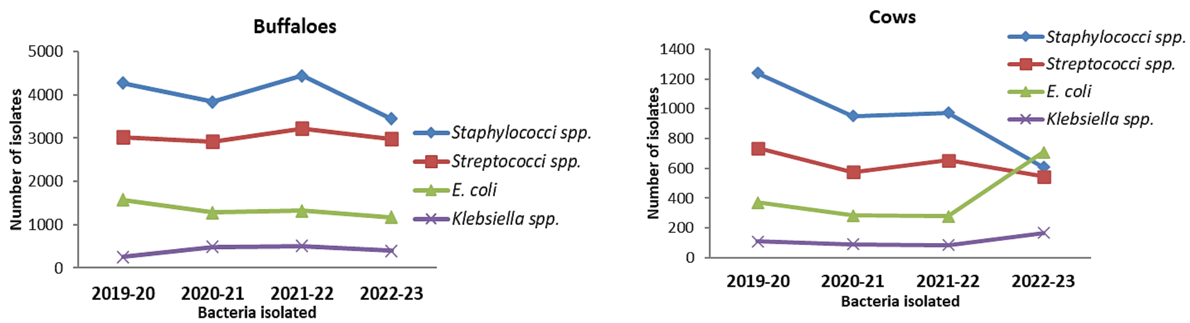

Fig. 2. Major pathogens isolated from bovine subclinical mastitis cases

The major Gram positive bacterial pathogens associated with mastitis in the entire study period were found as Staphylococcus species from both the cows and buffaloes with an average incidence rate of 45.53% and 44.1%, respectively. The Streptococcus species were found with 33.76% and 29.94% of total isolates from buffaloes and cows, respectively. Among Gram negative bacteria, E. coli was the most predominant bacteria isolated (17.37% and 13.85%) followed by Klebsiella spp. (5.54% and 5.19%) from both the cows and buffaloes, respectively. The major mastitis pathogens isolated annually from clinical and subclinical cases of bovines were shown in Figs 1 and 2, respectively. Almost similar trend was observed in the entire study period, except that the Gram negative isolates were shown an increase in isolation rate from subclinical cases of cows towards the later years of study.

Staphylococci and *Streptococci* were listed as the two most prevalent bacterial pathogens that can cause mastitis among the various microbial causal agents across the globe [8,9]. These are well known as contagious mastitis pathogens. *Staphylococcus*, *Streptococcus* and coliforms were reported as commonest etiological pathogens of mastitis from India also [3,26,27]. *Staphylococcus* species, *Streptococcus* species and *Escherichia coli* each had a pooled prevalence estimate of 45%, 13% and 14%, respectively in the metanalysis study from India [19]. There were no changes observed in the dominance of etiological agents of mastitis in bovines of Haryana State also. *Staphylococcus* and *Streptococcus* isolates were found as the predominant isolates in this study. However, the current study revealed that the annual proportion of contagious pathogens was significantly increased from both the cows and buffaloes affected with mastitis.

Escherichia coli and *Klebsiella* were recognized as the environmental mastitis pathogens [28,29]. An increase in the environmental pathogens was observed among cows affected with both clinical and subclinical mastitis. This may be due to the transmission of these pathogens to bovines during and between milking, dry period and calving time. The environmental factors like hygiene level at animal, udder and milking machine, milking practices, milker's hygiene and housing system plays crucial role in the occurrence of environmental mastitis [4].

3.2 Clinical Nature and Lactation Status of Mastitis Cases

It was found that, a significant proportion of clinical cases of mastitis were chronic in nature from both the species of bovines involved in this study. However, buffaloes (67.35%) were having a higher occurrence of chronic mastitis when compared to cows (59.28%) followed by sub acute (42.24% and 38.39%) and acute (34.64% and 27.57%) cases, respectively. There was no peracute case reported in this study. The analysis of clinical pattern of mastitis showed significant effect (p <0.05) with a chi-square value of 6.95 (Table 3).

The majority of mastitis cases were reported from first three parities. The highest incidences of mastitis was observed in the first lactation among buffaloes (20.69%) followed by third lactation (18.61%). Among cows, the maximum incidences of mastitis were observed in second and third lactation with 16.59% and 16.99%, respectively. The animals with lactation year above seven were also found to be more susceptible to mastitis. The impaired immune status due to high production of milk or old age can be correlated with this data. Similar reports were available from other parts of India [10, 30, 31]. However, some researchers reported bovines in the later stage of lactation period were more prone to mastitis [15, 32]. However, in both the species, maximum occurrence of mastitis was observed during the first lactational month (Fig. 3). The higher incidences during early lactation may be due to the oxidative stress and high production [26,33]. The chi-square analysis showed that, the occurence of mastitis with respect to different lactation number and lactation months were significant at $p < 0.05$ (Table 4). This is in accordance with the findings of other researchers from Asian countries [26,34,35].

Table 3. Clinical nature of mastitis

Type	Cows		Buffaloes		$\chi 2$	*P* value
	n=3366	Percentage	n=7563	Percentage		
Acute	764	22.69	1892	25.07		
Sub acute	1038	30.84	2287	30.24	6.95	0.03
chronic	1564	46.46	3384	44.74		

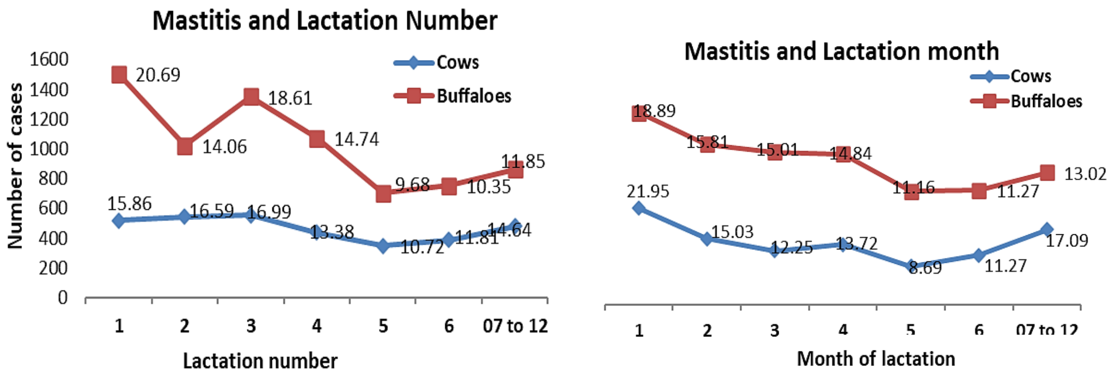

Fig. 3. Proportional prevalence of mastitis during different lactation number and lactation month

Table 4. Incidence of mastitis in different lactation number and months

Species	Lactation number						
	1	**2**	**3**	**4**	**5**	**6**	**7-12**
Cow (n=3266)	518	542	555	437	350	386	478
Buffalo (n=7263)	1503	1021	1352	1071	703	752	861
χ2	41.41 (*P* value < 0.00001)						
Species	**Lactation month**						
	1	**2**	**3**	**4**	**5**	**6**	**7-12**
Cow (n=3266)	717	491	400	448	284	368	558
Buffalo (n=7566)	1429	1196	1136	1123	844	853	985
χ2	34.99 (*P* value< 0.00001)						

Table 5. In-vitro per cent drug sensitivity of *Staphylococcus* isolates from bovines

Antibiotics	Cows				Buffaloes			
	2019-20	**2020-21**	**2021-22**	**2022-23**	**2019-20**	**2020-21**	**2021-22**	**2022-23**
Amikacin	27.72	22.91	25.37	35.5	46.52	24.14	27.7	32.93
Cloxacillin	18.22	15.66	21.66	23.96	11.81	14.34	17.33	16.95
Gentamicin	44.98	45.59	52.75	56.77	51.15	45.64	51.78	58.32
Neomycin	18.18	13.84	26.77	37.82	19.64	14.77	22.29	39.67
Streptomycin	7.71	3.52	4.79	11.73	7.73	2.96	5.8	12.01
Amoxicillin	13.68	11.43	16.83	15.43	10.75	10.24	15.3	14.02
Ampicillin	13.71	14.65	16.9	17.4	10.79	10.69	15.19	15.13
Penicillin	8.96	9.84	13.4	15.7	6.7	7.67	13.36	13.43
Cefoperazone	19.82	22.79	30.31	44.34	17.21	20.31	34.43	42.03
Ceftriaxone	33.9	35.44	37.87	34.38	25.08	30.95	34.23	33.77
Chloramphenicol	34.86	30.08	29.58	48.32	36.18	33.56	31.09	44.09
Enrofloxacin	38.87	41.79	54.19	55.53	44.6	42.64	55.76	58.43
Levofloxacin	33.29	27.29	28.21	32.72	37.07	30.86	27.97	32.4
Moxifloxacin	43.33	36.71	28.5	35.97	48.71	41.03	31.76	40.75
Oxytetracycline	17.54	9.92	8.21	9.91	15.41	7.8	7.58	9.86

Table 6. In-vitro per cent drug sensitivity of *Streptococcus* isolates from bovines

Antibiotics	Cows				Buffaloes			
	2019-20	**2020-21**	**2021-22**	**2022-23**	**2019-20**	**2020-21**	**2021-22**	**2022-23**
Amikacin	24.64	18.4	22.44	29.53	27.35	20.75	27.43	31.21
Cloxacillin	16.55	12.67	18.22	16.78	11.36	12.32	16.78	15.48
Gentamicin	48.43	43.33	53.14	53.13	52.11	45.24	53.54	56.33
Neomycin	16.06	12.4	27.4	35.47	19.95	12.96	29.1	37.31
Streptomycin	6.31	2.93	3.91	8.52	7.05	1.74	5.72	10.81
Amoxicillin	15.02	10	14.61	14.18	10.66	8.77	16.26	13.29
Ampicillin	15.38	13.26	14.55	16.37	10.76	11.24	15.91	13.98
Penicillin	14.59	7.67	12.11	15.07	6.51	6.18	13.34	12.94
Cefoperazone	19.49	19.27	36.88	42.49	17.34	18.12	35.43	41.7
Ceftriaxone	31.08	32.2	37.12	31.37	24.88	30.33	34.7	32.72
Chloramphenicol	31.39	26.6	26.85	48.22	35.43	32.21	30.42	43.71
Enrofloxacin	19.05	38.6	52.41	55.45	45.07	40.94	56.68	57.2
Levofloxacin	29.06	23	27.58	30.35	34.47	2.88	33.83	29.48
Moxifloxacin	45.8	35.13	30.88	38.19	51.61	39.01	33	38.11
Oxytetracycline	14.4	6.4	6.91	8.11	14.27	6.02	7.58	8.92

3.3 *In vitro* Antimicrobials Sensitivity Testing

All the isolates obtained from cows and buffaloes were subjected to *in vitro* antimicrobial sensitivity testing. The major Gram positive bacterial isolates responsible for mastitis of bovines were found as *Staphylococcus* spp. and *Streptococcus* spp. and their sensitivity pattern are given in Table 5 and Table 6, respectively. *E. coli* and *Klebsiella* were the predominant Gram negative bacteria isolated from this study. The antibiotic sensitivity patterns of them were shown in Table 7 and Table 8, respectively. It was found that, most of the samples were sensitive for enrofloxacin and gentamicin and there was an increase in the sensitivity of these antibiotics towards the later years of the study period. The penicillins group of betalactam antibiotics was given least sensitivity in this study, when compared to other classes of antibiotics. However, a reduction in sensitivity was observed for moxifloxacin and oxytetracycline against the isolates obtained from bovines during later years of study.

A wide variation in the antibiotic susceptibility spectrum has been observed throughout the world against the mastitogens. These variations are depending on many factors like animal nutrition and health status/immunity, management practices, geographical and climatic differences, prevention and control programs adopted. Studies from USA reported susceptibility towards ampicillin, cephalothin and ceftiofur for *S. aureus* and *S. dysgalactiae* isolates but are resistant to tetracycline. The proportion of resistant isolates was relatively high among Gram negative isolates [26]. Reports from Romania observed a resistance to aminoglycosides, macrolides and tetracyclines and susceptibility to penicillins and quinolones among Gram negative bacteria [36]. The *S. aureus* isolates from African countries were found as susceptible to gentamicin, ciprofloxacin, erythromycin, sulphamethoxazole/trimethoprim and chloramphenicol and high level of resistance to penicillin and tetracycline [37]. However, rare resistance to antibiotics was observed in Australian dairy herds [38]. The researchers from Asian countries reported a similar antibiogram of mastitis pathogens as that of our study. A higher susceptibility towards gentamicin and enrofloxacin and resistance against oxytetracycline against bacterial agents of mastitis in bovines from Pakistan was reported [39], while norfloxacin was found as most effective antibiotic in Lahore [40].

Table 7. In-vitro per cent drug sensitivity of *E. coli* isolates from bovines

Antibiotics	Cows				Buffaloes			
	2019-20	**2020-21**	**2021-22**	**2022-23**	**2019-20**	**2020-21**	**2021-22**	**2022-23**
Amikacin	25.2	19.34	21.13	25.34	25.2	19.34	21.13	25.34
Cloxacillin	2.33	1.19	0.89	2.77	2.33	1.19	0.89	2.77
Gentamicin	51.81	46.06	51.46	59.01	51.81	46.06	51.46	59.01
Kanamycin	5.13	4.7	5.74	5.93	5.13	4.7	5.74	5.93
Neomycin	13.89	9.11	19.82	32.47	13.89	9.11	19.82	32.47
Streptomycin	4.5	1.24	1.22	4.83	4.5	1.24	1.22	4.83
Amoxicillin	2.75	1.69	1.91	2.67	2.75	1.69	1.91	2.67
Ampicillin	2.68	1.97	2.32	3.73	2.68	1.97	2.32	3.73
Penicillin	1.46	0.49	1.95	3.39	1.46	0.49	1.95	3.39
Cefoperazone	7.47	9.48	18.11	29.45	7.47	9.48	18.11	29.45
Ceftriaxone	16.06	22.3	24.75	20.42	16.06	22.3	24.75	20.42
Chloramphenicol	24.75	13.64	20.96	25.01	24.75	13.64	20.96	25.01
Enrofloxacin	43.29	39.09	54.56	56.33	43.29	39.09	54.56	56.33
Levofloxacin	34.91	29.89	32.53	29.6	34.91	29.89	32.53	29.6
Moxifloxacin	40.11	31.71	21.09	24.77	40.11	31.71	21.09	24.77
Oxytetracycline	8.41	1.73	1.5	2.34	8.41	1.73	1.5	2.34

Table 8. In-vitro per cent drug sensitivity of *Klebsiella* isolates from bovines

Antibiotics	Cows				Buffaloes			
	2019-20	**2020-21**	**2021-22**	**2022-23**	**2019-20**	**2020-21**	**2021-22**	**2022-23**
Amikacin	31.16	19.38	18.7	24.21	23.45	16.13	16.84	26.82
Cloxacillin	0	1.36	0	0	0.85	0.61	0.58	0
Gentamicin	51.36	55.1	53.87	64.73	52.82	46.76	49.26	64.4
Kanamycin	39.72	33.67	28.7	3.15	41.09	3.49	1.85	5.19
Neomycin	11.98	7.48	15.8	23.68	9.24	7.81	12.85	29.91
Streptomycin	2.73	0	0.97	0.526	1.71	1.02	0.38	2.47
Amoxicillin	0	0.68	2.25	0.526	0.25	0.71	0.29	0.37
Ampicillin	0	0.34	1.61	0.526	0.85	0.51	0.38	0.98
Penicillin	0	0	0.97	1.57	0.25	0	0.48	1.11
Cefoperazone	6.16	9.86	11.93	22.63	5.39	6.47	11.1	31.39
Ceftriaxone	16.43	22.1	19.03	26.31	10.44	22.19	22	23.73
Chloramphenicol	21.57	20.74	19.03	17.89	19.43	20.45	16.55	22.37
Enrofloxacin	40.41	49.65	51.61	70	42.63	40.95	53.45	62.67
Levofloxacin	36.3	26.53	14.51	48.94	37.58	33.81	33.49	32.88
Moxifloxacin	3.08	3.74	3.87	32.1	37.67	31.34	14.6	28.05
Oxytetracycline	6.84	2.38	0.64	0.53	4.36	0.2	0.29	0.86

There are many reports of antibiogram studies against mastitis causing pathogens in bovines with similar and different findings from India. Enrofloxacin as the most sensitive drug against mastitis pathogens in bovines of Eastern Haryana and Tamil Nadu [11, 22] and 100% sensitivity to ciprofloxacin and gentamicin from Kashmir valley [3] was reported. The isolates from bovine clinical mastitis of Jammu shown maximum sensitivity to enrofloxacin and gentamicin while least sensitivity to oxytetracycline [27]. The Gram negative isolates obtained from cattle of West Bengal with subclinical mastitis were reported with tetracycline resistance [12]. Quinolones was reported as most efficacious drug against *Staphylococcus* from bovine mastitis of Madhya Pradesh [41], while ceftriaxone and amoxicillin-salbactum with maximum resistance and levofloxacin with high sensitivity against *Staphylococcus* isolates from bovines of Gujarat [42]. In another study, gentamicin was found as the most effective antibiotic followed by enrofloxacin from Meerut [43]. *S. aureus* isolates of mastitis from Maharashtra given highest resistance towards cephalexin [44]. Penicillin and streptomycin was reported as the most resistant antibiotics and chloramphenicol and ceftriaxone with high sensitivity against Gram positive bacteria and chloramphenicol and gentamicin for *Klebsiella* and *E. coli* isolates associated with bovine mastitis from Haryana [45]. The maximum antimicrobial resistance for amoxicillin-sulbactam and ceftriaxone from bovines of Gujarat against *Streptococcus* and gentamicin was least resistant was observed [46]. The Gram negative pathogens from Southern Haryana were found as sensitive to chloramphenicol, enrofloxacin, amikacin and ampicillin, while ceftizoxime and amoxicillin were shown maximum resistance [13].

Our laboratory is continuously make efforts in spreading the awareness among dairy entrepreneurs about the need of discriminate use of antibiotics whenever the mastitis occurs among bovines. The samples are usually referred to our lab once the curability of mastitis at the field conditions was impaired. The University is providing the facility for culture and sensitivity test for the farmers with subsidized rates. This encourages the farmers for submitting the samples to know about the pathogens and their sensitivity pattern. It is quite evident from the increase in the annual proportion of samples submitted to the laboratory. This data of mastitis causing pathogens and their sensitivity pattern in Hisar and adjoining districts of Haryana helps the veterinarians to adopt for the proper treatment protocols and management decisions for controlling mastitis, thus ensuring the prudent use of antibiotics.

4. CONCLUSIONS

The occurrence of specific mastitis etiological agents in different years and their antibiotic sensitivity were compared in this investigation. It was found that the proportion of pathogens causing mastitis was increased over the analyzed years from 2019-2023. *Staphylococcus*, *Streptococcus* and *Escherichia coli* were the major bacterial isolates obtained in this study. Environmental bacteria have gradually taken on more of a role when compared to infectious pathogens in subclinical cases. The antibiotics enrofloxacin and gentamicin showed higher sensitivity to the bacterial isolates obtained. The investigations pertaining to etiological agents help to adopt proper and targeted preventive programmes against mastitis The knowledge regarding the pattern of sensitivity of antibiotics enables the veterinarians and dairy farmers to assure optimal health, welfare and productivity of bovines in the State. This facilitates the efforts of the ongoing control programs and preventive measures against mastitis, thus reducing AMR to the maximum possible level based on one health approach.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jamali H, Barkema HW, Jacques M, Lavallee-Bourget EM, Malouin F, Saini V, Stryhn H, Dufour S. Incidence, risk factors and effects of clinical mastitis recurrence in dairy cows. J Dairy Sci. 2018;101:4729-4746. Available:https://doi.org/10.3168/jds.2017-13730.
2. Cobirka M, Tancin V, Slama P. Epidemiology and classification of mastitis. Animals (Basel). 2020;10:2212. Available:https://doi.org/10.3390/ani10122212.
3. Akhoon ZA, Peer FU, Nazir A. Epidemiology of mastitis in Kashmir valley. Indian J Comp Microbiol Immunol Infect Dis. 2015;36:43-45.

4. Argaw A. Review on epidemiology of clinical and subclinical mastitis on dairy cows. Food Sci Qual Manag. 2016;52:56-65.
5. Shaheen M, Tantary HA, Nabi SU. 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. 2016;4:150.
6. IDF. Guidelines for defining quarter and udder health status and cured clinical and subclinical mastitis cases. In: Bulletin of the IDF No. 515/2022. International Dairy Federation (ed.), Brussels; 2022.
7. Zigo F, Vasil M, Ondrasovicova S, Vyrostkova J, Bujok J, Pecka-Kielb E. Maintaining optimal mammary gland health and prevention of mastitis. Front Vet Sci. 2021;8. Available:https://doi.org/10.3389/fvets.2021.607311.
8. Acharya KR, Brankston G, Slavic D, Greer AL. Spatio-temporal variation in the prevalence of major mastitis pathogens isolated from bovine milk samples between 2008 and 2017 in Ontario, Canada. Front Vet Sci. 2021;8:742696. Available:https://doi.org/10.3389/fvets.2021.742696.
9. Naranjo-Lucena A, Slowey R. Antimicrobial resistance in bovine mastitis pathogens: A review of genetic determinants and prevalence of resistance in European countries. J Dairy Sci. 2023;106:1-23. Available:https://doi.org/10.3168/jds.2022-22267.
10. Tufani NA, Makhdoomi DM, Hafiz A. Epidemiology and therapeutic management of bovine mastitis. Indian J Anim Res. 2012;46:148-151.
11. Chandrasekaran D, Nambi AP, Thirunavukkarasu PS, Venkatesan P, Tirumurugaan KG, Vairamuthu S. Incidence of resistant mastitis in dairy cows in Tamil Nadu, India. J Appl Nat Sci. 2015;7:304-308.
12. Das A, Guha C, Biswas U, Jana PS, Chatterjee A, Samanta I. Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal, Vet World. 2017;10:517-520.
13. Manoj J, Singh MK. Seasonal prevalence and antibiogram studies of bovine mastitis in Southern Haryana. J Anim Res. 2020;10:1037-1042.
14. Dougnon V, Chabi Y, Koudokpon H, Agbankpe J, Sefounon R, Alle D, Bankole H, Baba-Moussa L. 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. 2020;6:34-40.
15. Swami SV, Patil RA, Gadekar SD. Studies on prevalence of subclinical mastitis in dairy animals. J Entomol Zool Stud. 2017;5:1297-1300.
16. Quinn PJ, Markey BK, Leonard FC, Fitz Patrick ES, Fanning S, Hartigan PJ. Veterinary microbiology and microbial diseases, Wiley Blackwell, West Sussex, UK. 2011;115-292.
17. CLSI. Performance standards for antimicrobial susceptibility testing standards. 30th ed., CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
18. Bangar YC, Verma MR, Dohare AK, Mukherjee R. Meta-analysis of prevalence of clinical mastitis in crossbred cows in India (1995-2014). J Anim Res. 2016;6:933-938. Available:https://doi.org/10.5958/2277-940X.2016.00133.9.
19. Krishnamoorthy P, Suresh KP, Saha S, Govindaraj G, Shome BR, Roy P. Meta-analysis of prevalence of subclinical and clinical mastitis, major mastitis pathogens in dairy cattle in India. Int J Curr Microbiol App Sci. 2017;6:1214-1234. Available:https://doi.org/10.20546/ijcmas.2017.603.141.
20. Krishnamoorthy P, Goudar AK, Suresh KP, Roy P. Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. Res Vet Sci. 2021;136:561-586. Available:https://doi.org/10.1016/j.rvsc.2021.04.021.
21. Kumari T, Bhakat C, Choudhary RK. A Review on sub clinical mastitis in dairy cattle. Int J Pure App Biosci. 2018;6:1291-1299. Available:http://dx.doi.org/10.18782/2320-7051.6173.
22. Bhanot V, Chaudhri SS, Bisla RS, Singh H. Retrospective study on prevalence and antibiogram of mastitis in cows and buffaloes of Eastern Haryana. Indian J Anim Res. 2012;46:160-163.

23. Dua K. Incidence aetiology and estimated loss due to mastitis in India- An update. Indian Dairy Man. 2001;53:41-48.
24. Mourya A, Shukla PC, Gupta DK, Sharma RK, Nayak A, Singh B, Jain A, Pradhan S. Prevalence of subclinical mastitis in cows in and around Jabalpur, Madhya Pradesh. J Entomol Zool Stud. 2020;8:40-44.
25. Devi M, Dutta JB. Incidence of bovine subclinical mastitis in organized and unorganized farms based on somatic cell count. Int J Chem Stud. 2018;6:1399-1403.
26. Sharma N, Rho GJ, Hong YH, Kang TY, Lee HK, Hur TY, Jeong DK. Bovine mastitis: An Asian perspective. Asian J Anim Vet Adv. 2012;7:454-476.
27. Bhat AM, Soodan JS, Singh R, Dhobi IA, Hussain T, Dar MY, Mir M. Incidence of bovine clinical mastitis in Jammu region and antibiogram of isolated pathogens. Vet World. 2017;10:984-989.
28. Belay N, Mohammed N, Seyoum W. Bovine mastitis: Prevalence, risk factors and bacterial pathogens isolated in lactating cows in Gamo Zone, Southern Ethiopia. Vet Med (Auckl). 2022;13:9-19. Available:https://doi.org/10.2147/VMRR.S344024.
29. Goulart DB, Mellata M. *Escherichia coli* mastitis in dairy cattle: etiology, diagnosis, and treatment challenges. Front Microbiol. 2022;13:928346. Available:https://doi.org/10.3389/fmicb.2022.928346.
30. Sharma N, Maiti SK, Sharma KK. Prevalence, Etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). Int J Dairy Sci. 2007;2:145-151. Available:https://doi.org/10.3923/ijds.2007.145.151.
31. Sharma N, Maiti SK. Incidence, etiology and antibiogram of sub clinical mastitis in cows in durg, Chhattisgarh. Indian J Vet Res. 2010;19:45-54.
32. Kurjogi MM, Kaliwal BB. Epidemiology of bovine mastitis in cows of Dharwad district. Int Sch Res Notices. 2014;968076. Available:http://dx.doi.org/10.1155/2014/968076.
33. Abdi RD, Gillespie BE, Ivey S, Pighetti GM, Almeida RA, Dego OK. Antimicrobial resistance of major bacterial pathogens from dairy cows with high somatic cell count and clinical mastitis. Animals. 2021;11:131. Available:https://doi.org/10.3390/ani11010131.
34. Fadlelmula A, Al Dughaym AM, Mohamed GE, Al Deib MK, Al Zubaidy A. Bovine mastitis: Epidemiological clinical and etiological study in a Saudi Arabian large dairy farm. Bulg J Vet Med. 2009;12:199-206.
35. Suresh M, Safiullah AHM, Kathiravan G, Narmatha N. Incidence of clinical mastitis among small holder dairy farms in India. J Vet Sci. 2017;12(1):1-13.
36. Pascu C, Herman V, Iancu I, Costinar L. Etiology of mastitis and antimicrobial resistance in dairy cattle farms in the western part of Romania. Antibiotics (Basel). 2022;11:57. Available:https:// doi.org/10.3390/antibiotics11010057.
37. Grima LYW, Leliso SA, Bulto AO, Ashenafi D. Isolation, identification and antimicrobial susceptibility profiles of *Staphylococcus aureus* from clinical mastitis in Sebeta Town Dairy Farms. Vet Med Int. 2021; 1772658. Available:https://doi.org/10.1155/2021/1772658.
38. Dyson R, Charman N, Hodge A, Rowe SM, Taylor LF. A survey of mastitis pathogens including antimicrobial susceptibility in southeastern Australian dairy herds. J Dairy Sci. 2022;105:1504-1518. Available:https://doi.org/10.3168/jds.2021-20955.
39. Ali T, Kamran, Raziq A, Wazir I, Ullah R, Shah P, Ali MI, Han B, Liu G. Prevalence of mastitis pathogens and antimicrobial susceptibility of isolates from cattle and buffaloes in northwest of Pakistan. Front Vet Sci. 2021;8:746755. Available:https://doi.org/10.3389/fvets.2021.746755.
40. Imran M, Rehman I, Sulehria AK, Butt YM, Khan AM, Ziauddin A. Profile of antimicrobial susceptibility from cattles’s milk isolates suffering from mastitis in district Lahore. J Biores Manag. 2021;8:55-60. Available:https://doi.org/10.35691/JBM.1202.0180.
41. Baghel A, Chhabra D, Sharda R, Shukla S, Audarya S, Sikrodia R, Gangil R. Isolation of *Staphylococcus* from bovine mastitis and their antibiotic sensitivity pattern. Indian J Vet Sci Biotechnol. 2018;13:49-52.

42. Javia BB, Purohit JH, Mathapati BS, Barad DB, Savsani HH, Ghodasara SN, Kalariya VA, Patel UD, Nimavat VR. Molecular detection and antimicrobial resistance pattern of *Staphylococci* isolated from clinical and subclinical bovine mastitis. Indian J Vet Sci Biotechnol. 2018;14:13-16.
43. Verma H, Rawat S, Sharma N, Jaiswal V, Singh R. Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. J Entomol Zool Stud. 2018;6:706-709.
44. Yadav MM. Prevalence of *Staphylococcus aureus* in lactating cows with subclinical mastitis and their antibiogram in organized dairy farm, Maharashtra, India. Int J Curr Microbiol App Sci. 2018;7:3674-3680. Available:https://doi.org/10.20546/ijcmas.2018.703.425.
45. Mittal D, Sharma A, Singh M, Rajesh, Mahajan NK. Antimicrobial sensitivity pattern observed in microbes associated with bovine mastitis. Haryana Vet. 2018;57(2):215-218.
46. Javia BB, Mathapati B, Barad D, Ghodasara S, Savsani H, Bhadaniya A, Fefar DT, Patel UD, Sindhi SH. Bacteriological and molecular detection with antimicrobial resistance pattern of major *Streptococcus* spp. isolated from bovine mastitis. Int J Curr Microbiol App Sci. 2020;9:2443-2451.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113597