Original Research Article

A TEMPORAL STUDY ON INCIDENCE OF BOVINE MASTITIS IN HARYANA, INDIA

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 mastitis were chronic in nature from both the species of bovines. The incidence of mastitis with respect to different lactation number and lactation months wasfound 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.

Key words: Antimicrobial resistance, Bovines, Haryana, Mastitis, *Staphylococcus*, *E. coli*

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.

MATERIALS AND METHODS

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].

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.

***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).

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.

RESULTS AND DISCUSSION

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 3,990cows 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.

Table 1: The milk samples processed for bacteriological examination by culturalmethod

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**			

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 2: Annual proportion of bacterial isolates obtained from the milk samples of the studyMastitis 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**		

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 Fig.1 and 2, respectively. Almost similar trend was observed in the entire study period, except that the Gram negativeisolates were shown an increase in isolation rate from subclinical cases of cows towards the later years of study.

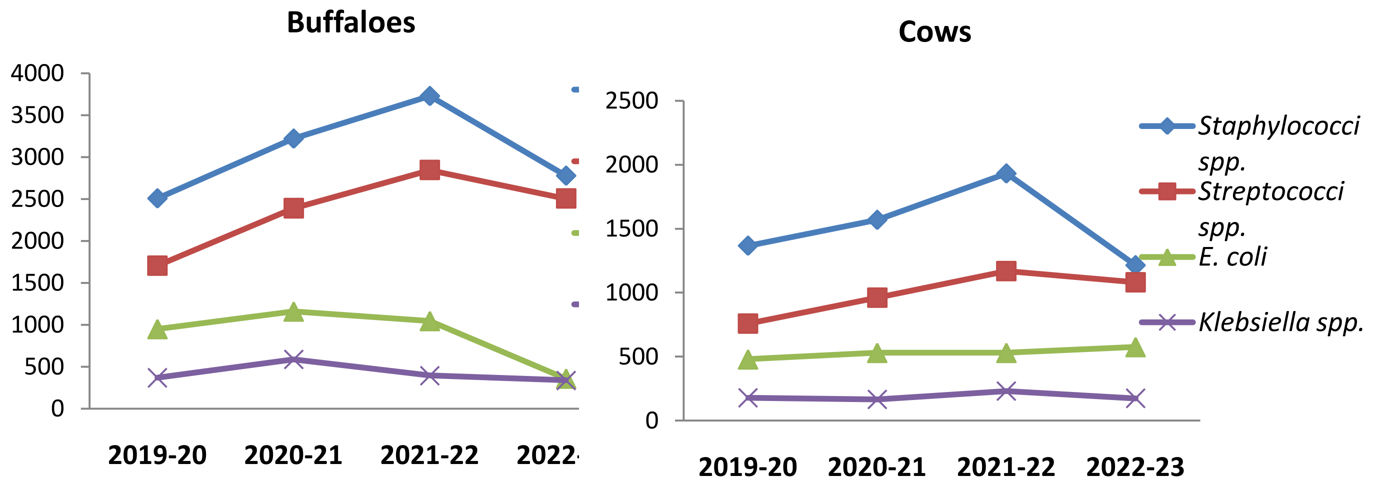

Fig 1: Major pathogens isolated from bovine clinical mastitis cases

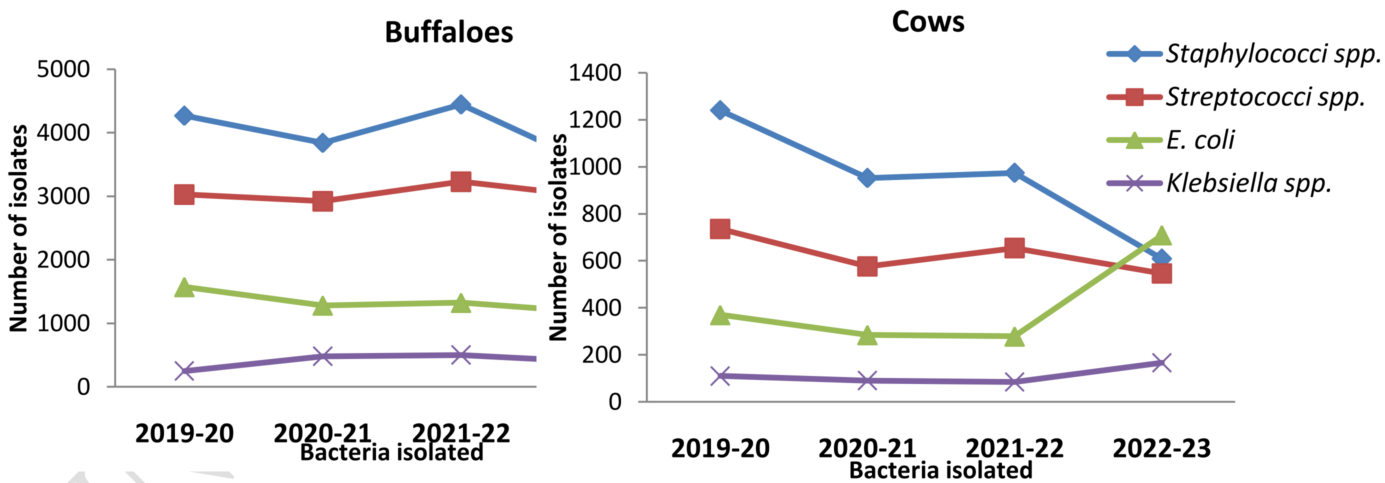

Fig 2: Major pathogens isolated from bovine subclinical mastitis cases

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].

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).

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	6.95	0.03
Sub acute	1038	30.84	2287	30.24		
chronic	1564	46.46	3384	44.74		

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, 32, 33]. However, some researchers reported bovines in the later stage of lactation period were more prone to mastitis [15, 34]. 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].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, 30, 31].

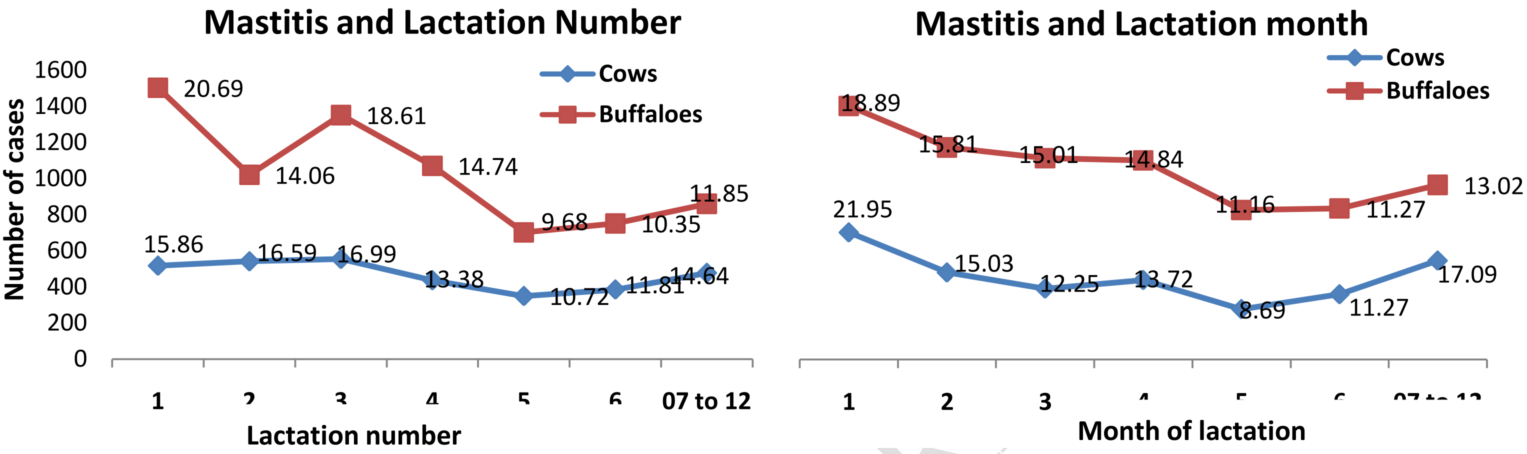

Fig.3: Proportional prevalence of mastitis during different lactation number and lactation month

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)						

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.

Table 5: In-vitro per cent drug sensitivity of *Staphylococcus* isolates frombovines

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 frombovines

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

Table 7: In-vitro per cent drug sensitivity of*E. coli*isolates frombovines

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 frombovines

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

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].

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.

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.

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