SciMedCentral
Journal of Veterinary Medicine and Research

Review Article

Prevalence of Subclinical Mastitis and Antibiogram of *Escherichia Coli* in Cow Milk of Western Chitwan

Koirala Aavash[1]*, Gyawali Sajita[1], Gyawali Chet Narayan[2], and Shah Anjay Kumar[2]

[1]*Livestock Development Officer, Ministry of Agriculture and Livestock Development, Nepal*

[2]*Veterinarian, Nepal Agricultural Research Council, Nepal*

***Corresponding author**

Koirala Aavash, Livestock Development Officer, Ministry of Agriculture and Livestock Development, Bagamati Province, Nepal, Tel: 9779855011921

Submitted: 03 August 2023

Accepted: 30 August 2023

Published: 31 August 2023

ISSN: 2379-948X

Keywords

- Subclinical mastitis
- CMT
- *E. coli*

Abstract

Mastitis, a management-related disease affecting cow production efficiency, was studied in 90 dairy cattle in Western Chitwan. Subclinical mastitis (SCM) prevalence was determined using the CMT test, with 31.09% (111 out of 357) of milk samples testing positive. No significant difference was found in quarter-wise prevalences of SCM. Commercial farms had a higher SCM prevalence (39%) than conventional farms, a statistically significant finding.

The CMT test demonstrated a sensitivity of 95% in identifying SCM. Bacteriological culture and biochemical tests revealed *E. coli* in 16.25% (18 out of 106) of samples. Among the antibiotics tested on Muller-Hilton Agar using CLSI 2012, Ciprofloxacin, Norfloxacin, and Tetracycline were most effective, while Amoxyclav was completely resistant. Furthermore, 83.33% of isolates displayed a Multiple Antibiotic Resistance (MAR) index exceeding 0.2, indicating the need for rational antibiotic use.

The study highlights a concerning SCM prevalence of 31.09% in Chitwan's dairy cattle, with 16.98% of SCM cases attributed to *E. coli*. These findings indicate emerging management issues affecting animal health and economic losses. The study emphasizes the importance of farm sanitation and personal hygiene to mitigate the risk of *E. coli* infection. Additionally, prudent antibiotic use and public awareness are crucial to control the unregulated antibiotic usage.

Overall, the study underscores the significance of managing mastitis in dairy cattle through effective practices and preventive measures. Reducing SCM prevalence can enhance production efficiency and economic outcomes for farmers in the region.

INTRODUCTION

Nepal, a developing country heavily reliant on agriculture, has approximately 65.6% of its population engaged in this sector. Agriculture contributes about 32% to the total GDP, with livestock playing a vital role. Traditionally, livestock farming in Nepal was predominantly sustainable, but commercialization has gradually modified this approach. The rise of commercial cattle farms in Chitwan has been notable, with 365 registered farms and several others seeking registration. Despite the economic importance of livestock, farmers often face challenges due to poor management practices and hygiene, resulting in reduced productivity. Mastitis, a prevalent disease worldwide, significantly affects dairy animal production and health, leading to decreased milk yield and higher somatic cell counts. Mastitis can be classified as clinical and subclinical.

Subclinical Mastitis in Cattle

In cattle, mastitis is associated with many different infectious agents, commonly divided into those causing contagious mastitis, which are spread from infected quarters to other quarters and cows; those that are normal teat skin inhabitants and cause opportunistic mastitis; and those causing environmental mastitis, which are usually present in the cow's environment and reach the teat from that source [1]. The environmental coliforms include the Gram-negative bacteria *E. coli*, *Klebsiella spp.,* and *Enterobacter spp. A. pyogenes* mastitis can be an important problem in some herds. Some pathogens involved in mastitis are Contagious pathogens: *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis,* and *Corynebacterium bovis* Teat skin opportunistic pathogens: *coagulase-negative staphylococci*, Environmental pathogens: environmental *Streptococcus spp.,* including *Streptococcus uberis* and *Streptococcus dysgalactiae*, which are the most prevalent; less prevalent is *Streptococcus equinus* (formerly referred to as *Streptococcus bovis*). Environmental coliforms include the Gram-negative bacteria *Escherichia coli, Klebsiella spp., Enterobacter spp.,* and *Arcanobacterium* (formerly *Actinomyces) pyogenes.* Uncommon pathogens: many, including *Nocardia spp., Pasteurella spp., Mycobacterium bovis, Bacillus cereus, Pseudomonas spp., Serratia marcescens, Citrobacter spp.*, anaerobic bacteria species, fungi, and yeasts. A total of about 140 microbial species, subspecies, and serovars have been isolated from the bovine mammary gland [1].

Detecting subclinical mastitis requires special diagnostic tests since there are no visible milk abnormalities. The somatic cell count (SCC) is commonly used to identify inflammatory changes, with a higher SCC indicating greater tissue inflammation. And bacteriological culture can also identify the pathogens involved in the development of subclinical mastitis [2]. This problem is worldwide. Etiologies for mastitis are like bacteria, mycoplasma, fungus, and viruses, but among them, bacteriological origins are the most common. The sources of infection included an infected cow, contaminated bedding and manure, vaginal and uterine infections, the milker's hand, a suckling calf, the milking machine, and house flies [2].

Many articles have claimed that the worldwide prevalence of SCM is high and is a major concern regarding the prevention of mastitis. If we can treat or decrease the incidence of SCM, then we can minimize the risk of mastitis. Many studies have been done in different countries to calculate the prevalence of SCM, and some of the findings are: in Chitwan, the prevalence of SCM was found to be 33.33% [3]. An Epidemiological investigation of subclinical bovine mastitis in western Chitwan, Nepal, by Dhakal [4], has found a 30% prevalence of SCM in cattle. Khakural [5], found 17.2% of SCM in the Kathmandu Valley.

Shrestha and Bindari [6], analyzed 200 milk samples collected from 50 dairy cows in Bhaktapur. They found 52% of animals suffering from subclinical mastitis. A study was conducted by Sudhan [7], to determine the prevalence of sub-clinical mastitis and the pathogen associated with sub-clinical mastitis in India. His findings suggest that the prevalence of SCM is 14.43% in cattle.

E. coli in Cattle with SCM

E. coli is responsible for the development of coliform infections, both clinical and subclinical. They are generally found in bedding, manure, and the digestive tract and cause environmental mastitis. Many studies have been done to find out the prevalence of *E. coli* in SCM. Shrestha and Bindari [6], analyzed 200 milk samples collected from 50 dairy cows in Bhaktapur. They isolated 10% *E. coli* from the total bacterial growth of a subclinical mastitis-positive sample.

A similar study done by Hamal found that 6.89% of the *E. coli* in the sample was positive for SCM. Sudhan [7], found 1.72% *E. coli* in an SCM-positive sample. And this research was done on organized farms only. A study conducted by Hameed et al. [8], in Pakistan to study microorganisms associated with mastitis in cattle found *E. coli* (16%).

Pathogenesis of E. coli Mastitis

Coliform bacteria, such as *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae*, and *Serratia marcescens*, are common pathogens responsible for causing mastitis [9]. These bacteria are natural inhabitants of soil, digestive tracts, manure, and bedding materials. Contaminated bedding, with coliform numbers reaching 1,000,000 or more per gram, increases the likelihood of udder infections and clinical mastitis.

Coliform bacteria invade the udder through the teat sphincter when the teat ends come into contact with them. Once inside the mammary gland, coliform bacteria can either rapidly multiply or remain dormant. As the cow's immune system attempts to destroy them, coliforms release endotoxins into the cow's body. These endotoxins cause changes in vascular permeability, leading to edema and acute swelling of the gland, as well as a significant increase in neutrophils in the milk. The concentration of neutrophils may increase 40–250 times, effectively inhibiting the survival of *E. coli*. This excessive migration of neutrophils is linked to the pronounced systemic leukopenia and neutropenia seen in cases of peracute coliform mastitis [1]. Clinical signs of coliform mastitis are primarily caused by the endotoxins produced by the bacteria. Infected cows display a high fever, a depressed appetite, rapid weight loss, abnormal milk production, and decreased milk production. Seasonal patterns, such as high temperatures, heavy rainfall, and unstable weather conditions, often contribute to new clinical infections. Severe cases are more common in older, high-producing cows early in lactation. Coliform bacteria are widespread in the environment, affecting all dairy herds to varying degrees. While they cause a high percentage of acute clinical cases, they account for less than 5% of total infected quarters within a herd at any given time. In some cases, the release of sufficient endotoxin can lead to seriously ill cows and even death.

Coliform bacteria cause numerous cases of acute clinical mastitis in dairy cows. Affected cows exhibit high fever, udder inflammation, depressed appetite, dehydration, diarrhea, decreased milk production, and abnormal milk. The milk may appear watery with clots, but these characteristics do not necessarily indicate the specific mastitis pathogen. Typically, only one quarter of cows are clinically infected, although coliforms can also cause persistent subclinical infections. Treating these infections is generally not effective, as the majorities are eliminated by the cow's immune system.

Antibiotic Sensitivity Test for *E. coli*

Treatment of coliform mastitis in cattle has been controversial, as when *E. coli* is destroyed by the cow's immune system, it releases a toxin (endotoxin) called lipopolysaccharide endotoxin, which is the primary cause for the development of clinical signs. Antibiotics act to kill the bacteria, and in this case, these infections would result in the production of endotoxin, which is fatal to cow health [10]. But sometimes the infection becomes systemic and severe; in such cases, the administration of antibiotics through the perenteral route, followed by an intramuscular infusion, fluid therapy, and electrolyte therapy, is recommended [1].

Unregulated uses of antibiotics lead to the development of

Extended Spectrum beta-lactamase enzymes producing *E. coli (ESBL in E. coli)* [11], and multiple antibiotic-resistant strains of *E. coli*. Hence, proper drug selection and antibiotic sensitivity testing should be performed before prescribing antibiotics.

In a study done by Chandrasekaran et al. [12], on the treatment of resistant mastitis in dairy cows, 86.65% of isolated *E. coli* were found to be resistant, i.e., resistant to 1 or 2 antimicrobials, and only a few *E. coli* isolates (13.45%) were found to be multi-drug resistant, i.e., resistant to 3 or more antimicrobials.

Antibiotics have been beneficial in enhancing growth, performance, and treating ailments in the dairy industry. However, treatment is often administered only after cows exhibit clinical signs without early screening tests or proper diagnosis, leading to haphazard antibiotic use. Subclinical mastitis is difficult to detect due to the absence of any visible indications and has major cost implications associated with decreased milk production [13]. In India, the annual economic loss to the dairy industry due to subclinical mastitis is estimated to be Rs. 43653 million [14]. The incidence of coliform mastitis has increased since serious efforts have been made to eliminate *Staphylococcus aureus* and *Streptococcus sp.* Mastitis [15]. Recently, due to the unregulated use of various antimicrobial agents without AST, antibiotic resistance strains of pathogens have been developed, like MRSA [16], and ESBL-producing *E. coli*. And these strains are transmitted to humans through the ingestion of infected milk and have public health concerns.

Objectives

- To identify subclinical mastitis using an indirect test, i.e., the California Mastitis Test
- Determine the sensitivity of the California mastitis test
- Isolate and phenotypically identify coli from cattle with subclinical mastitis.
- Antibiotic sensitivity test to find out the choice of drugs against *E. coli*.

METHODS AND MATERIALS

The research was carried out in Geetanagar, Rampur, and Shardanagar of Chitwan district. All these areas were in similar condition and contained significant cattle populations in both conventional and commercial farming systems. A cross-sectional study was conducted in the study area for the determination of the prevalence of subclinical mastitis and the antibiogram of *E. coli* in cow milk from September, 2017 to December 2017. The cattle milk under study was basically from conventional and commercial farms. A farm having more than 10 livestock units was defined as a commercial farm, and up to 10 large cattle units are considered conventional farms [17]. 357 samples from animals of different farms were taken. Among 90 cattle, 50 cattle from commercial farm and remaining from individual farm were selected purposively. And all the microbiological lab works were done in National Cattle Research Program microbiology lab.

The teats were swabbed with 70% ethyl alcohol and allowed to dry. Initial milk streaks were discarded. Milk was then collected aseptically in sterile vials labeled as fore right, fore left, hind right, and hind left. The samples were used in the laboratory within an hour. Subclinical mastitis detection involved using a subclinical mastitis detector and comparing milk values with the reference range for subclinical mastitis. A California mastitis test was also performed for subclinical mastitis identification.

Microbiological Analysis of Sample

Culture of Milk Sample: The infected milk samples were streaked on nutrient agar and MacConkey agar, followed by overnight incubation at 37°C. Petri plates with no microbial growth after incubation were further incubated for 48 hours. Gram staining of colonies on nutrient agar was performed to identify gram-negative bacteria. The gram-negative bacteria exhibiting grayish-white colonies on nutrient agar and rose-pink colonies on MacConkey agar were subcultured on EMB agar at 37°C for 24 hours. Biochemical tests, including the indole test, the methyl red test, the Voges-Proskauer test, the citrate utilization test, and the oxidase test, were conducted using the gram-negative colonies from nutrient agar. The identification of *E. coli* was based on the following characteristics: large, smooth, opaque, or partially translucent moist greyish-white colonies on nutrient agar; rose-pink colonies on MacConkey agar; metallic sheen seen on EMB agar; positive indole and methyl red tests; negative Voges-Proskauer test; citrate test; and oxidase test.

Antibiotic Sensitivity Test: By Kirby-Bauer disc diffusion method using Mueller-Hinton agar plate following guidelines provided by the CLSI [18]. Briefly, 0.5 McFarland of bacterial suspension was inoculated on Muller Hilton Agar and following disk were placed: Gentamycin (10mg), Ciprofloxacin (5mg), Norfloxacin (10mg), Tetracycline (30mg), Cefotoxime (30mg) and Amoxyclav (30mg).

Data Analysis

- Data analysis was done using SPSS version 16.0.
- The association between different variables was analyzed using the Chi square test at a 5% level of significance.
- The MAR index was calculated from the AST data. The MAR index of an isolate is defined as a/b, where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was subjected [19].

RESULTS

Prevalence of Subclinical Mastitis

Early screening tests were done to identify subclinical mastitis, and out of 357 milk samples, 111 were identified as SCM. Among the 357 samples, 246 (68.90%) showed CMT negativity. 69 (19.32%) showed mild positive (+) and 42 (11.76%) showed strong positive (++). CMT in at least one quarter, but without clinical symptoms, was classified as SCM-positive. So the quarter-wise prevalence of SCM was found to be 31.09%. This has been illustrated in Figure 1.

Quarter-wise prevalence of SCM

The prevalence of SCM in the left front, right front, left hind, and right hind was found to be 34.44%, 26.14%, 33.33%, and 30.34%, respectively. The highest prevalence was found in the left half. There was no significant difference in the quarter-wise prevalence of SCM (P< 0.05). This has been illustrated in Figure 2.

Farming system-wise prevalence of SCM

(Table 1) In my study prevalence of subclinical mastitis was high in commercial farming system which was found to be 39% than conventional (20.6%) which was highly significant (P<0.05).

Sensitivity of CMT in response to bacterial growth

Out of 111 positive milk samples, only 106 showed bacterial growth on bacteriological culture. The remaining five samples did not show any bacterial growth. Hence, the sensitivity of the CMT test in response to bacterial growth was found to be 95% (Figure 3).

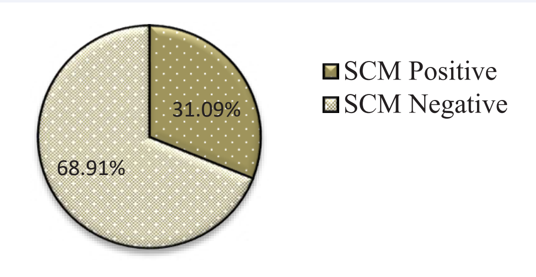

Figure 1 Prevalence of SCM in cow milk.

Table 1: Farming system-wise prevalence of SCM

Farming system	SCM results		OR(CI)	χ^2	P value	Result
	Positive n(%)	Negative n(%)				
Commercial	78(39%)	122(61%)	2.461 (1.53-3.96)	14.073	0.000	Highly significant
Conventional	33(20.6%)	127(79.4%)				

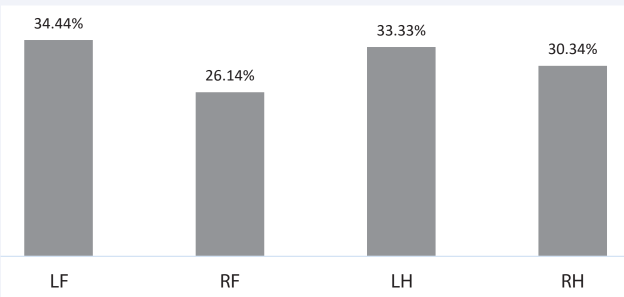

Figure 2 Quarter wise prevalence of SCM.

Proportion of E. coli in Cow Milk with SCM

Out of 106 bacterial cultures, *E. coli* was isolated in 18 colonies through colony morphology and biochemical tests. Hence, the proportion of **E. coli** in cow milk with SCM was found to be 16.98%, which is given in the pie chart below (Figure 4).

Antibiotic Sensitivity Results of Isolates *E. coli*

In my study *E coli* was more sensitive to Ciprofloxacin, Norfloxacin and Tetracycline with 100% sensitivity. Amoxyclav was found to be complete resistance. The graphical representation of sensitivity pattern of different antibiotics against *E. coli* isolates is given below (Figure 5).

Multiple Antibiotic Resistant Index of Isolated *E. coli*

In this study, 6 different antibiotics were used and MAR index was calculated for each *E coli* isolated. The graphical representation of MAR indices of individual bacterial isolates against 6 different antibiotics is shown in Figure 6.

Here, the maximum isolates showed the MAR index of 0.2 and 83.33% isolates showed MAR index more than 0.2.

DISCUSSION

In a study of mastitis in Western Chitwan District, researchers using the CMT found the prevalence of SCM to be 30% in cows [4], which was similar to the present research findings. Based on

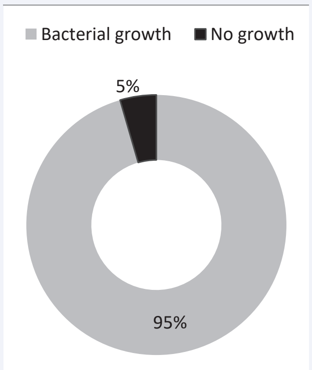

Figure 3 Sensitivity of CMT in response to bacterial growth.

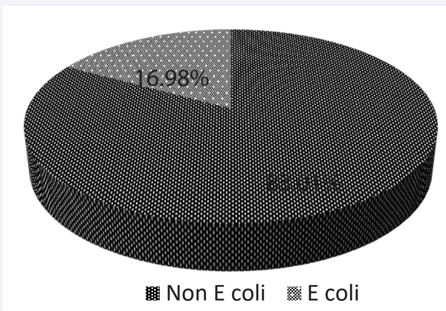

Figure 4 Pie chart showing proportion of E. coli in cow milk with SCM.

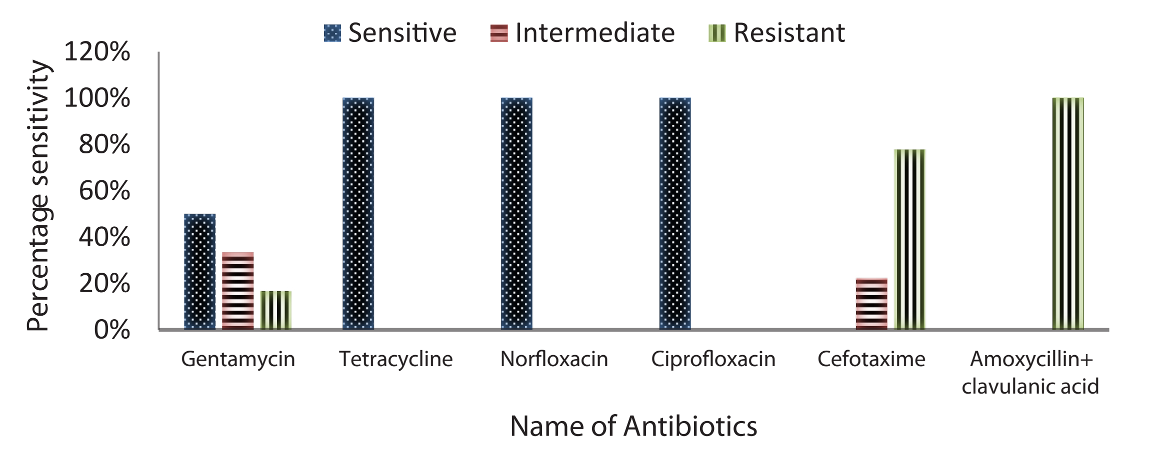

Figure 5 Bar graph showing AST results of isolates *E. coli*.

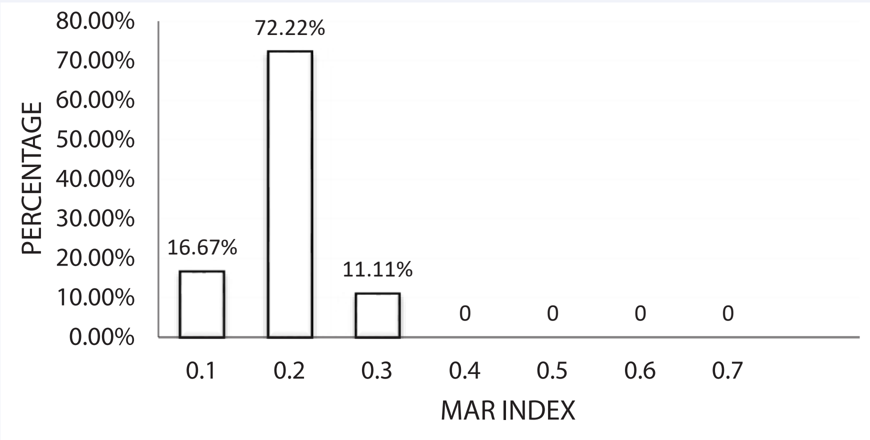

Figure 6 Bar graph showing MAR indexing of E. coli isolates on 6 different antibiotics.

CMT, the overall prevalence of SCM was 33.33% [3], in Chitwan, another similar finding.

In contrast to my findings, Shrestha and Bindari [6], found a 52% prevalence of SCM in Bhaktpur, Nepal, on the basis of CMT, which was higher than my findings and could be due to the different management practices used by those farmers. They also explained that their result of a higher prevalence was due to poor management practices. Poor hygiene and milking practices are reported to accelerate the disease [20].

Also, the findings of Sudhan [7], which were contradicted and lower than my findings, which are about 15.62%, the appropriate reason for such a difference, might be due to the seasonality of the research, which was done on organized farms only.

In my study, the prevalence of subclinical mastitis was higher in commercial farming systems than conventional ones, which was statistically significant ($P<0.05$). This could be due to the high number of animals on commercial farms, and the chance of infection spreading from one animal to another is likely to be high. However, Rahman [21], in Bangladesh found no significant difference among farming systems ($P>0.05$).

Quarter-wise, the occurrence of SCM was generally high in the left quarters. Statistically, there was no significant difference in the quarter-wise prevalence of SCM ($P>0.05$) which was similar to the findings of Shittu et al. [22], in Nigeria and Hashemi et al. [23], in Iran. Though an immediate explanation cannot be established for this observation, it is highly likely that in the process of milking, these particular quarters were milked first before the other quarters because most of the operators tend to be right-handed and sit first with the left animals [19].

Out of 111 positive milk samples, only 106 showed bacterial growth on bacteriological culture. My finding was similar to the findings of Saidi [24], who found 96% sensitivity of CMT in response to bacterial growth, and Teklesilasie [25], who found 97.6% sensitivity.

The higher prevalence rate of *E. coli* in cow milk with SCM on the basis of bacteriological culture and biochemical properties found in the research of Hameed et al. [8], which was 16% in Pakistan, was similar to my findings. And also, the research done by Hashemi et al. [23], (13.64% in Iran) was in agreement with my findings.

However, Sudhan [7], found a prevalence of 1.72% of *E. coli* in SCM, which was much lower than my findings despite the same procedure. The reason might be due to the different geographical location, climatic conditions during sampling, and hygiene practices of the study area. Also, Hamal [26-30], found a 6.89% prevalence of *E. coli* in SCM in Chitwan district, which was lower than my findings, which might be due to different climatic conditions and a small sample size [31-35].

E. coli was more sensitive to Ciprofloxacin, norfloxacin, and Tetracycline. Resistance to Cefotaxime and Amoxyclav might be due to the high use of beta-lactam antibiotics to treat mastitis in cattle. Research done by Chandrasekaran et al., [12, 36-45], found 86.65% isolated *E.coli* were found to be resistant i.e resistance to 1 or 2 of antimicrobials and few *E. coli* isolates (13.45 %) were found to be multi-drug resistant i.e. resistance to 3 or more of antimicrobials which is similar to my findings. The reason might be due to the unregulated use of antibiotics or the prescription of drugs without AST that develop multiple antibiotic resistance strains [46-51].

CONCLUSION AND RECOMMENDATIONS

My research findings indicate a high prevalence of subclinical mastitis, highlighting its emergence as a significant managemental problem impacting animal health and causing economic losses. Coliform mastitis, caused by the environmental pathogen *E. coli*, is linked to poor hygiene and management practices. The study revealed a notable difference in infection rates among different farming systems, with commercial farming showing a higher prevalence. However, no significant variation was observed in quarter-wise occurrences of SCM. The sensitivity of 95% for CMT suggests its use as an early screening test for identifying SCM. The high proportion of *E. coli* in milk with subclinical mastitis underscores the emerging management challenges. The unregulated use of antibiotics without antibiotic susceptibility testing (AST) has led to an alarming increase in multiple antibiotic-resistant strains, posing a severe threat to veterinary and public health.

RECOMMENDATIONS

- An antibiotic sensitivity assay should be performed before prescribing antibiotics.
- Emphasis on farm sanitation and personal hygiene to reduce the chance of coliform infection spreading.
- Regular use of early screening tests (CMT) should be done to identify the SCM so that proper prevention measures can be applied before it turns into a clinical infection.

REFERENCES

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary medicine: A textbook of the disease of cattle, sheep, goat and horse. 10th edn. London: BailliereTindall. 2006; 680-715.
2. Chakrabarti A. A textbook of preventive veterinary medicine. 5th Ed. Kalyani Publishers India. 2007.
3. Sharma P. Prevalence, Antibiogram and risk factors associated with Staphylococcus aureusin cow milk of Chitwan district. Master thesis of AFU. 2015.
4. Dhakal IP and KR Tiwari. Epidemiological investigation of subclinical bovine mastitis in western Chitawan of Nepal. Journal of Institute of Agriculture and Animal Science (IAAS), Research Reports. 1993; 427-434.
5. Khakural GP. Study on prevalence of subclinical mastitis in Kathmandu valley. Proceedings of the 1st National Workshop on Livestock and Fisheries Research in Nepal, Khumaltar, Lalitpur. 1996; 185-188.
6. Shrestha S and YR Bindari. Prevalence of sub-clinical mastitis among dairy cattle in Bhaktapur District, Nepal. Inter J Agri Biosci. 2012; 1: 16-19.
7. Sharma N, V Pandey, NA Sudhan, Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. Bulg J Vet Med. 2010; 13: 98–103.
8. Hameed S, M Arshad, M Ashraf, M Avais and MA Shahid. Prevalence of common mastitogens and their antibiotic susceptibility in Tehsil Burewala, Pakistan. Pak J Agri Sci. 2008; 2011: 45.
9. Ruegg P L. California Mastitis Test (CMT) Fact Sheet 1 California Mastitis Test (CMT) Fact Sheet. 2005; 1: 16–18.
10. Petersson-wolfe CS, Science D, Tech V. Escherichia coli : A Practical Summary for Controlling Mastitis. Virginia cooperative extension. 2011.
11. Kaşikçi G, Çetİn Ö, Bİngöl EB, and Gündüz MC. Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows. Turk J Vet Anim Sci. 2012; 36: 49–55.
12. Chandrasekaran D, Nambi AP, Thirunavukkarasu PS, Vairamuthu S, and Venkatesan P. A study on treatment of resistant mastitis in dairy cows. J Appl Nat Sci. 2014; 6: 786 – 791.
13. Abdel-rady A. Epidemiological Studies on Subclinical Mastitis in Dairy cows in Assiut Governorate. Veterinary World. 2009; 2: 373-380.
14. Sargeant, KE Leslie, JE Shirley, BJ Pulkrabek, and GH Lim. Sensitivity and Specificity of Somatic Cell Count and California Mastitis Test for Identifying Intramammary Infection in Early Lactation. J Dairy Sci. 2001; 84: 2018-2024.
15. Stalberger RJ and Kersting KW. Peracute Toxic Coliform Mastitis. Iowa State University Veterinarian. 1988; 50: 1.
16. Hussein SA. Prevalence and Bacterial Etiology of Subclinical Mastitis in Dairy Cows in Al Sulaimaniyah District. Kufa J Vet Sci. 2012; 3: 1.
17. Dieser SA, Vissio C, Lasagno MC, Bogni CI, Larriestra AJ. Prevalence of Pathogens Causing Subclinical Mastitis in Argentinean Dairy Herds. Pak Vet J. 2014; 34: 124-126.
18. Aal AEl. Correlation between some direct and indirect tests for screen detection of subclinical mastitis. Int Food Res J. 2014; 21: 1249-1254.
19. Ali Ghulam Muhammad, Maira Arshad, Muhammad Saqib. Bacteriology of mastitis in buffaloes in tehsil samundri of district Faisalabad, Pakistan. Pak Vet J. 2008; 28: 31-33.
20. Pankey JW, RJ Elberhart, AL Cuming, RD Daggett, RJ Farnworth and CK McDuff. Update on Post Milking Teat Antisepsis. J Dairy Sci. 1984; 67: 1336.

21. Rahman MA, SMA Rauf and MU Ahmed. Prevalence of mastitis in cows as dairy and subsistence farming systems. J Agrofor Environ. 2012; 6: 51-55.

22. Shittu, Jamilu Abdullahi, Aliyu Jibril, Aminu A Mohammed, and Folorunso O Fasina. Sub-clinical mastitis and associated risk factors on lactating cows in the Savannah Region of Nigeria. BMC Vet Res. 2012; 8:134.

23. Hashemi M, M Kafi, M Safdarian. The prevalence of clinical and subclinical mastitis in dairy cows in the central region of Fars province, south of Iran. Iranian Journal of Vet Res. 2011; 12: 3.

24. Saidi R, Khelef D, Kaidi R. Bovine mastitis : Prevalence of bacterial pathogens and evaluation of early screening test. Afr J Microbiol Res. 2013; 7: 777–782.

25. Teklesilasie T, Kebeta MM, Fayera T. Bovine Mastitis : Prevalence and Isolation of Major Pathogens in Dairy Farms of Selected Sites in Addis Ababa, Ethiopia. Appl Ind Hyg. 2014; 3: 31–37.

26. Galfi A, and Milanov D. Electrical conductivity bacteriological findings subclinical mastitis. Biotechnology in Animal Husbandry. 2015; 31: 533-541.

27. Ayano AA, Hiriko F, Simyalew AM, and Yohannes A. Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. J Vet Med Anim Health. 2013; 5: 67-72.

28. Chakraborty P. A textbook of Microbiology. 3rd Ed. New central book agency (p) ltd. India. 2013; 308-313

29. Danielt RCW, Biggst DA, Barnum DA. The relationship between california mastitis test scores and monthly milk production and composition. Can Vet J. 1996; 7: 99-105.

30. Dingwell Randy, Leslie Ken, Timms Leo L, Schukken Ynte, Sargent. Evaluation of the California Mastitis Test to Determine Udder Health Status of Early Lactation Dairy Cows, Animal Industry Report. 2004; 650: R1913.

31. Ganguly S, Qadri K, and Kumar P. Bacteriological analysis of mastitic milk sample. IJMRME. 2016; 1: 1.

32. Idriss, V Foltys, V Tančin, K Kirchnerová, D Tančinová, K Zaujec. Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra, Slovakia. Slovak J Anim Sci. 2014; 47: 33-38.

33. Ilie LI, Tudor L, and Galiş AM. The electrical conductivity of cattle milk and the possibility of mastitis diagnosis in Romania. Vet Med Sci. 2010; 43

34. Joe Hogan, K Larry Smith. Coliform mastitis. Vet Res. 2003; 34: 507-519.

35. Lien C, Wan Y, Ting C. Engineering in Agriculture, Environment and Food Online detection of dairy cow subclinical mastitis using electrical conductivity indices of milk. Eng Agric Environ Food. 2016; 9: 201–207.

36. Mbindyo, George Gitao, Lily Caroline Bebora. A cross-sectional study on the prevalence of subclinical mastitis and antimicrobial susceptibility patterns of the bacterial isolates in milk samples of small holder dairy goats in Kenya. Am J Res Commun. 2014; 2.

37. Mcfadden M. California Mastitis Test and Milk Quality. Michigan dairy review. 2011; 16: 1–3.

38. Murugaiyah Marimuthu, Faez Firdaus Jesse A, Konto Mohammed, Sangeetha Sarvananthan, Yusuf Abba, Lawan Adamu. Prevalence and antimicrobial resistance assessment of subclinical mastitis in milk samples from selected dairy farms. Am J Anim Vet. 2014; 9: 65-70.

39. Reneau JK. Somatic cell counts: measures of farm management and milk quality. National Mastitis Council Annual Meeting Proceedings. 2007; 29: 29–37.

40. Rice Duane N. G81-556 Using the California Mastitis Test (CMT) to Detect Subclinical Mastitis. Historical Materials from University of Nebraska-Lincoln Extension. 2008; 483.

41. Salvador RT, Garcia AAA. Correlation of California mastitis test and somatic cell count on milk of water buffalo cows in the Philippines. Trop Agric. 2014; 90: 3

42. Sanothoran, Mylvaganam Pagthinathan. Prevalence of Bovine Subclinical Mastitis and its Association with Bacteria and Risk Factors in Milking Cows of Batticaloa District in Sri Lanka. IJSRIT. 2016; 3: 6.

43. Sayed RH, Salama SS, Soliman RT. Bacteriological Evaluation of Present Situation of Mastitis in Dairy Cows. Global Vet. 2014; 13: 690-695.

44. Seegers H, Fourichon C, Beaudeau F. Production Effects Related to Mastitis and Mastitis Economics in Dairy Cattle Herds. Vet Res. 2003; 34: 475-491.

45. Shpigel, M Winkler, G Ziv, A Saran. Clinical, bacteriological and epidemiological aspects of clinical mastitis in Israeli dairy herds. Prev Vet Med. 1998; 35: 1–9

46. Singh D, Kumar S, Singh B, Bardhan, D. Economic losses due to important diseases of bovines in central India. Vet World. 2014; 7: 579–585.

47. Sumathi BR, Veeregowda BM, Gomes AR. Prevalence and antibiogram profile of bacterial Isolates from clinical bovine mastitis. Vet World. 2008; 237–238.

48. Suojala L, Kaartinen L, S Pyörälä. Treatment for bovine Escherichia coli mastitis – an evidence-based approach. J vet Pharmacol Therap. 2013; 36: 521-531.

49. Sylejmani D, Ramadani N, Robaj A, Hamidi A. Prevalence and antimicrobial susceptibility of bacterial isolates from subclinical mastitis in dairy farms in kosovo. Bulg J Vet Med. 2016; 19: 299-307.

50. Thapa S. Detection of subclinical mastitis and trends of antibiotic sensitivity in cattle of lelevdc, lalitpur. Int Rep. 2015.

51. Union E. Subclinical mastitis and associated risk factors on dairy farms in New South Wales. Aus Vet J. 2011; 89: 41–46.