Haryana Vet. (December, 2023) **62(2),** 24-28 **Research Article**

MOLECULAR DETECTION OF BACTERIA ASSOCIATED WITH THE SUBCLINICAL MASTITIS IN CATTLE AND THEIR ANTIBIOGRAM

R. SINGATHIA*, D.K. SHARMA, A. SAIN, K. RATHORE, K. PUROHIT[1], S.K. SHARMA[2] and M. JOSHI[3]
Department of Veterinary Microbiology, [1]Department of Veterinary Pathology,
[2]Department of Veterinary Clinical Medicine, [3]Department of Animal Nutrition,
College of Veterinary and Animal Science, Vallabhnagar, Udaipur, RAJUVAS, Rajasthan, India

Received: 15.02.2023; Accepted: 28.05.2023

ABSTRACT

The present study was undertaken to identify bacteria isolated from the milk samples collected from cattle affected with subclinical mastitis and their antibiogram profiling. A total of 769 quarter milk samples were screened for subclinical mastitis from 200 cattle in and around Udaipur city. The positive milk samples based on CMT and SCC were subjected to isolation of bacteria. After that, all these bacterial isolates were subjected to *in vitro* drug sensitivity testing. A total of 120 bacterial pathogens were isolated from 115 culturally positive quarters. Among 115 quarters, 110 (95.65%) quarters showed infection by a single bacterial species and 05 (4.35%) quarters showed mixed bacterial infection of *Staphylococcus* spp. + *Streptococcus* spp. Out of 115 organisms isolated, 71 were Staphylococci spp., 39 were Streptococci spp. and 10 were *E. coli*. So, Staphylococci were found to be the predominant organisms followed by Streptococci and *E. coli*. The antimicrobial sensitivity of isolates varied in different farms which depend on the use of antimicrobials and strains prevalent at that farm. Most strains of Staphylococci, Streptococci and *E. coli* were found sensitive to amikacin, chloramphenicol and gentamicin.

Keywords: *E. coli*, Staphylococci, Streptococci, Subclinical mastitis

How to cite: Singathia, R., Sharma, D.K., Sain, A., Rathore, K., Purohit, K., Sharma, S.K. and Joshi, M. (2023). Molecular detection of bacteria associated with the subclinical mastitis in cattle and their antibiogram. *Haryana Vet.* **62(2)**: 24-28.

Mastitis is an inflammatory response in the mammary gland, which is predominantly a result of the infectious challenge and is the most frequent and costly disease of dairy animals (Fonseca *et al.*, 2015). The subclinical form of the disease is important because it is 15 to 40 times more prevalent than its clinical form (Singh *et al.*, 2015) and therefore usually persists longer in the herd, causing production losses (Charaya *et al.*, 2014; Kumar *et al.*, 2014; Ali *et al.*, 2015). Subclinical mastitis (SCM) causes a direct loss of 6.8% animal-wise and 34.5% quarter-wise milk production and an indirect loss by reduced reproductive efficiency (Karthikeyan *et al.*, 2016). Most of the cases of worldwide SCM are caused by *Staphylococcus* spp., *Streptococcus* spp. and *E. coli* (El-Jakee *et al.*, 2013; Sunagar *et al.*, 2013; Charaya *et al.*, 2014; Singh *et al.*, 2014; Karthikeyan *et al.*, 2016; Ferdaus *et al.*, 2019; Maciel-Guerra *et al.*, 2021). Microbiological and somatic cell count (SCC) testing in milk is the most sensitive method for the measurement of infection of bovine mammary glands. The prevalence of SCM and organisms association varies from region to region and among animals on different farms. To avoid the problem of the emergence of antibiotic-resistant bacteria and to initiate an effective treatment of mastitis, *in vitro* antimicrobial sensitivity testing of mastitogenic isolates is recommended. Keeping in view the above-stated facts about subclinical mastitis, the present study was planned to determine the etiological agent(s) responsible for the causation of subclinical mastitis in cattle and to determine their antimicrobial sensitivity to institute proper line treatment and adoption of control measures.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted at the College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur.

A total of 769 milk samples were collected from quarters of 200 apparently healthy lactating cows collected in and around Udaipur City and screened for sub-clinical mastitis by using the California mastitis test (CMT) and SCC test.

Bacteriological examination

The positive milk samples on the basis of CMT and SCC were subjected to bacteriological examination. All the milk samples showing SCC greater than 5×10^5 cells/ml were subjected to isolation of bacteria and phenotypic characterization of bacterial isolates as per the standard techniques (Markey *et al.*, 2013).

Molecular detection of bacterial isolates

DNA extraction from bacterial culture isolates

The chromosomal DNA of Staphylococci and Streptococci from all the field isolates was extracted according to Wilson (1987) with slight modifications. The *E. coli* genomic DNA isolation was carried out by the heat treatment method as described (Li *et al.*, 2017). The purity and concentration of the DNA were estimated in a UV

*Corresponding author: drrajeshsingathia@gmail.com

absorbance biospectrophotometer (Eppendorf, Hamburg, Germany). The purity of the DNA was verified by measuring absorbance at 260 nm and 280 nm. A 260/280 ratio of approximately 1.8 was considered pure for DNA and were further used for the molecular assays.

Polymerase chain reaction (PCR) assay

The oligonucleotide sequences and the corresponding amplicon sizes for the identification of bacteria by PCR have been mentioned in Table 1. All the PCR tests for the identification of bacteria were carried out in a final volume of 25 µl. Each polymerase chain reaction (PCR) mixture consisted of 12.5 l of 2x mater mix (Genetix Biotech Asia Pvt. Ltd., New Delhi, India), 2.5 l of template DNA, 0.75 l (50pM) of forward primer, 0.75 (50pM) of reverse primer, 8.5 l of nuclease free water (NFW) (Genetix Biotech Asia Pvt. Ltd., New Delhi, India) in 25 l PCR reaction mix.

All Staphylococcal isolates were tested for carriage of 16S *rDNA* as described by Strommenger *et al.* (2003), whereas all Streptococcal isolates were tested for carriage to the *tuf* gene as per the method of Hegde (2011). All *E. coli* isolates were confirmed by species-specific PCR assay primers targeting the universal stress protein A (*uspA*) gene as described by Chen and Griffiths (1998). The primers were synthesized by Eurofins Genomics India Pvt. Ltd. (Bangalore, India). The DNA of *Staphylococcus*/*Streptococcus*/*E.coli* (standardized and maintained in the department of Veterinary Microbiology, CVAS, Navania) and NFW were used as positive and negative controls, respectively in each run and amplification was performed in Thermocycler (Biorad Pvt. Ltd., California, USA) with the following thermal cycle conditions for all the three primers used: initial denaturation at 94º C for 5 min, 30 cycles of denaturation at 94º C for 1 min, annealing (annealing temperature as described in table 1 for each primer) for 1 min, extension at 72º C for 1 min and final extension at 72º C for 10 min and held at 4º C.

PCR products were electrophoresed in 1.5% agarose gel to observe their consistency. The amplified product was visualized as a band of expected size under UV light and documented by a gel documentation system (Biogen Scientific, Cambridge, U.S.A.)

***In vitro* drug sensitivity pattern**

All the organisms isolated from udder infections were subjected to *in vitro* drug sensitivity testing, using 15 antimicrobial agents *viz.* amikacin, ampicillin, cefixime, cefotaxime/cephotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, co-triamoxazole, erythromycin, gentamicin, methicillin, ofloxacin, penicillin-G, streptomycin and tetracycline by Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) according to the Clinical and Laboratory Standards Institute's guidelines (CLSI, 2020). The sensitivity was observed based on the zone size interpretation chart, provided by the manufacturer. The results were recorded as sensitive, intermediate and resistant according to CLSI guidelines (2020).

RESULTS AND DISCUSSION

The overall quarter-wise prevalence based on CMT and SCC was 31.73% (244/769) and 20.02% (154/769), respectively as previously reported in our research paper (Singathia *et al.*, 2022). 14.95% (115/769) of the quarters were showing SCC above 500,000/ml of milk and were culturally positive.

During the cultural examination, a total of 120 organisms were isolated from 115 culturally positive quarters. A total of three genera including *Staphylococcus*, *Streptococcus* and *E. coli* were isolated in the present study. *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli* were further confirmed by PCR targeting 16S *rDNA* (Fig. 1), *tuf* (Fig. 2) and *uspA* (Fig. 3) genes, respectively. The results of the present study in terms of molecular detection of these isolates are consistent with the finding of other researchers (El-Jakee *et al.*, 2013; Sunagar *et al.*, 2013; Charaya *et al.*, 2014; Singh *et al.*, 2014), who also confirmed these isolates by PCR. Among 115 quarters, 110 (95.65 %) quarters showed infection by a single bacterial species and 05 (4.35 %) quarters showed mixed bacterial infection of *Staphylococcus* spp. + *Streptococcus* spp.

In the present study, Staphylococci were the most prevalent organism, accounting for 59.17% of the isolates followed by Streptococci (32.5%) and *E. coli* (8.33%). In this study, contagious bacteria like Staphylococci and Streptococci caused most of the infections. It may be attributed to unhygienic milking practices and that might have caused entry of these organisms into the mammary gland, through the milkers' hands, causing an increase in SCC and inflicting pathogenicity in the alveolar tissue. Further, spread of this infection from diseased animal to next animal at the time of milking is possible due to contagious nature of bacterial pathogens (Pankaj *et al.*, 2012).

These results were in accordance with Rani *et al.* (2008) who reported that amongst the various mastitogenic bacteria isolated, Staphylococci were the most prevalent, accounting for 67.99% and 63.62% of the infections in cows and buffaloes, followed by Streptococci (31.98% and 36.36%), respectively. Similar to the present observation, the high prevalence of Staphylococci has been reported by several researchers from India (Mittal *et*

Figs. 1 to 3. **(1)** Representative image of Mannitol fermentation by *Staphylococcus* on MSA after 24 hr incubation at 37º C; **(2)** Representative image of *Streptococcus* on Edward's Media after 24 hr incubation at 37º C; **(3)** Representative image of *E. coli* on Eosine methylene blue (EMB) after 24 hr incubation at 37º C

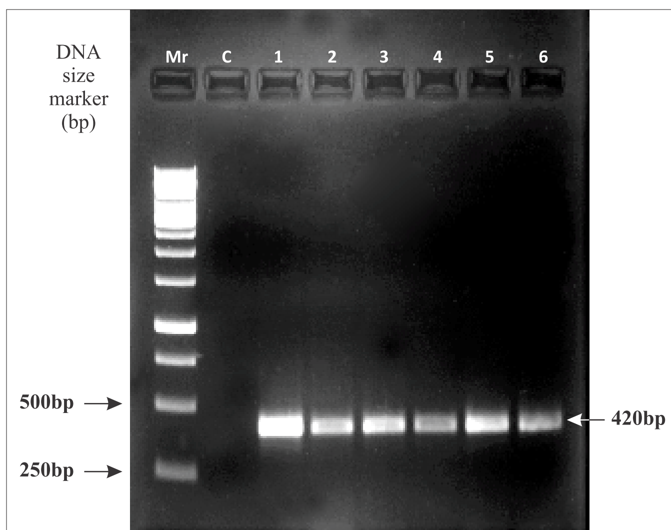

Fig. 4. Agarose gel electrophoresis of 16S *rDNA Staphylococcus genus* specific PCR products (420 bp) [Lane Mr: 250 bp DNA ladder, Lane C: Negative control, Lane 1-5: Farm isolates of *Staphylococci*, Lane 6: Positive control]

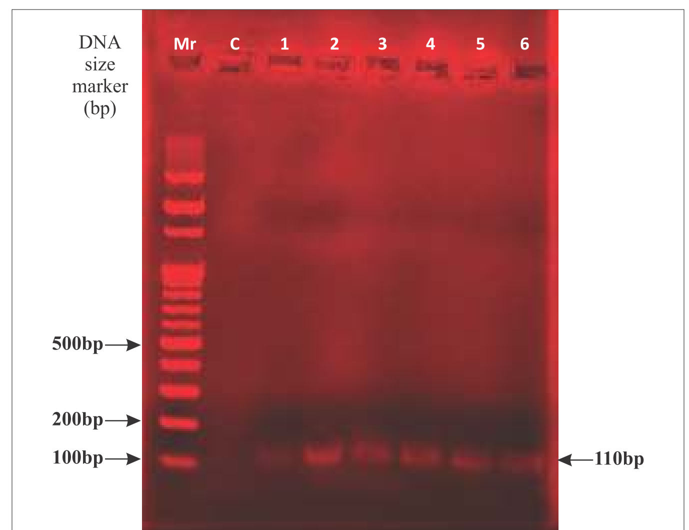

Fig. 5. Agarose gel electrophoresis of *tuf Streptococcus genus* specific PCR products (110 bp) [Lane Mr: 100 bp DNA ladder, Lane C: Negative control, Lane 1-5: Farm isolates of *Streptococci*, Lane 6: Positive control]

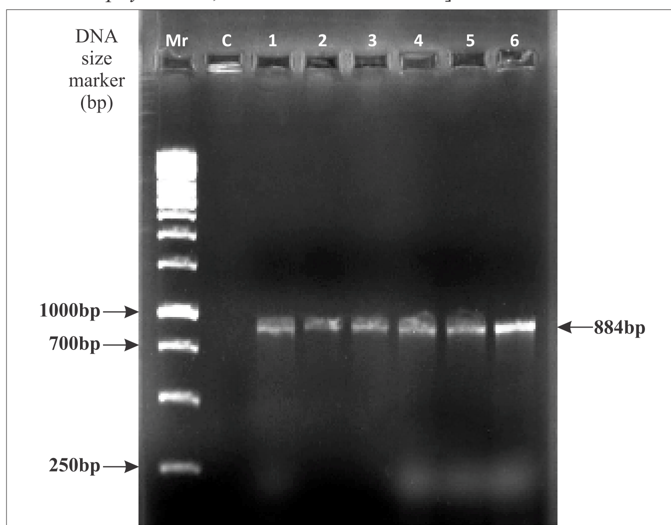

Fig. 6. Agarose gel electrophoresis of *uspA E. coli* specific PCR products (884 bp) [Lane Mr: 250 bp DNA ladder, Lane C: Negative control, Lane 1-5: Farm isolates of *E. coli*, Lane 6: Positive control]

al., 2018; Verma *et al.*, 2018; Solanki *et al.*, 2021) and abroad (Nickerson and Stephen, 2009; Tenhagen *et al.*, 2009).

Streptococci were the second most prevalent pathogen associated with cattle SCM in the present study which is in harmony with the findings of other researchers (Singh, 2015; Mittal *et al.*, 2018; Solanki *et al.*, 2021). While in contrast to our study Lakshmi and Jayavardhanan, (2016) reported *E. coli* as the second most prevalent organism.

E. coli was the third most prevalent pathogen (8.33%) associated with cattle SCM in the present study. The prevalence of *E. coli* as a major pathogen has been reported by several researchers (Singh *et al.*, 2016, Mittal *et al.*, 2018) and the prevalence reported by these workers ranged from 10.2 to 24.13 % in their studies. On the other hand, Awandkar *et al.* (2009) reported a higher incidence of *E. coli* infections (40.0%) in bovine mastitis.

Table 1. Details of oligonucleotide sequence used for detection of pathogen

Bacterial species and their gene	Sequence (52 - 32)	Ampliconsize (bp)	Annealing temperature (ºC)	Reference
Staphylococcus (16S *rDNA*)	F:5'-CAG CTC GTG TCG TGA GAT GT-3'R:5'-AAT CAT TTG TCC CAC CTT CG-3'	420	52ºC	Strommenger *et al.* (2003)
Streptococcus (*tuf*)	F:5'-CAA CTT GAC GAA GGT CCT GCA-3'R:5'-TGG GTT GAT TGA ACC TGG TTT A-3'	110	46ºC	Hegde (2011)
E. coli (*uspA*)	F:5'-CCGATACGCTGCCAATCAGT-3'R:-5'ACGCAGACCGTAGGCCAGAT-3'	884	49ºC	Chen and Griffiths (1998)

Staphylococci showed high sensitivity towards amikacin (100%), chloramphenicol (100%), gentamicin (100%), ciprofloxacin (94.44%), cefixime (94.11%), ofloxacin (94.11%), erythromycin (92.85%) and less sensitivity towards co-triamoxazole (83.33%), cefotaxime/cephotaxime (82.35%) and streptomycin (82.35%). The least sensitivity of Staphylococci was observed towards ceftriaxone (58.33%), ampicillin (47.05%), penicillin-G (40%), tetracycline (33%) and methicillin (31%). Overall, resistance was recorded against some of the antibiotics in Staphylococci isolated in the present study might be due to the extent of the use of antimicrobials for treatment and resistance strains of Staphylococci prevalent at that farm/area. Our results also concur with the finding of Sharma *et al.* (2015) who reported high sensitivity (88.89%) of Staphylococci towards Chloramphenicol and high resistance toward Penicillin. High sensitivity toward Fluoroquinolones in our study is in close agreement with those reported by Mohanty *et al.* (2013) and Mir *et al.* (2014).

Streptococci showed high sensitivity toward amikacin (100%), cefotaxime/cephotaxime (100%), ceftriaxone (100%), chloramphenicol (100%), gentamicin (100%), ofloxacin (93.75%), penicillin-G (100%), streptomycin (93.75%), and less sensitivity toward cefixime (80%), ciprofloxacin (76.47%), co-triamoxazole (85.71%), erythromycin (80%) and tetracycline (75%). The least sensitivity of Streptococci was observed toward ampicillin (42.85%) and methicillin (16.67%). Our findings corroborate with the finding of Pankaj *et al.* (2013) and Charaya *et al.* (2014), wherein the researchers showed high sensitivity of Streptococci towards ceftriaxone and gentamicin. *E. coli* showed high sensitivity toward chloramphenicol (100%), co-triamoxazole (100%), gentamicin (100%) and less sensitivity toward ciprofloxacin (88.89%), ofloxacin (88.89%), amikacin (81.82%), streptomycin (66.67%). The least sensitivity of *E. coli* was observed toward tetracycline (22.22%), cefixime (11.11%), cefotaxime/cephotaxime (11.11%), ceftriaxone (9.09%), ampicillin (0%), erythromycin (0%), methicillin (0%) and penicillin-G (0%).

The studies conducted by several researchers (Pankaj *et al.*, 2012; Mittal *et al.*, 2018; Solanki, 2021) have shown increased resistance to different traditional and newly introduced antibiotics. The emergence of these drug-resistant pathogens responsible for mastitis is due to the indiscriminate utilization of antibiotics.

In conclusion, the present study indicated a considerable occurrence of SCM and pathogens associated with SCM in and around Udaipur City of Rajasthan. Results of the present study indicate high levels of multidrug resistance which is matter of concern. Similar studies are also required at large scale so that appropriate treatment and control strategies should be formulated to eradicate or reduce the number of major pathogens which are associated with SCM. Therefore, continuous monitoring of AMR and application of AMR mitigation measures are required to control the spread of the infection to animals and humans. However, in the present study, the highest sensitivity was conferred to amikacin, chloramphenicol, and gentamicin which are suggestive of judicious use of these antibiotics in the treatment of bovine subclinical mastitis.

REFERENCES

Ali, Z., Dimri, U. and Jhambh, R. (2015). Prevalence and antibiogram of bacterial pathogens from subclinical mastitis in buffaloes. *Buff. Bullet.* **34(1)**: 41-44.

Awandkar, S.P., Khode, N.V., Sardar, V.M. and Mendhe, M.S. (2009). Prevalence and current antibiogram trend of mastitic agents in Udgir and its vicinity, Maharashtra state, India. *Int. J. Dairy Sci.* **4**: 117-22.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**: 493-496.

Charaya, G., Sharma, A., Kumar, A., Singh, M. and Goel, P. (2014). Pathogens isolated from clinical mastitis in Murrah buffaloes and their antibiogram. *Vet. World.* **7(11)**: 980-985.

Chen, J. and Griffiths, M.W. (1998). PCR differentiation of *Escherichia coli* from other Gram negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. *Lett. Appl. Microbiol.* **27(6)**: 369-371.

Clinical and Laboratory Standards Institute (2020). Performance standards for antimicrobial disk susceptibility test. M02 standard, (13th Edn.), Clinical and Laboratory Standards Institute,

Wayne. PA.

El-Jakee, J.K., Aref, N.E., Gomaa, A., El-Hariri, M.D., Galal, H.M., Omar, S.A. and Samir, A. (2013). Emerging of coagulase negative *Staphylococci* as a cause of mastitis in dairy animals: an environmental hazard. *Int. J. Vet. Sci. Med.* **1(2)**: 74-78.

Ferdaus, H., Anjuman, A., Mahfujur, R., Nabila, I., ATM, B., Eman, Z., Mukter, H., Ali, Z.M., Nazneen, A.R., Ashraful, I. and Masudur, R. (2019). Characterization of Bacterial Isolates, antibiogram profile and pro-inflammatory cytokines in subclinical mastitis in cross-bred dairy cows. *Alex. J. Vet. Sci.* **62(2)**: 1-10.

Fonseca, I., Cardoso, F.F., Higa, R.H., Giachetto, P.F., Brandão, H.M., Brito, M.A.V.P., Ferreira, M.B.D., Guimarães, S.E.F. and Martins, M.F. (2015). Gene expression proûle in zebu dairy cows (*Bos taurus indicus*) with mastitis caused by *Streptococcus* agalactiae. *Livest. Sci.* **180**: 47-57

Hegde, R. (2011). Rapid identification of bacterial pathogens causing subclinical bovine mastitis with special reference to *Staphylococcus aureus*, *Escherichia coli* and predominant Streptococcal species by molecular methods. (Doctoral Dissertation) Karnataka Veterinary, Animal and Fisheries Sciences University.

Karthikeyan, A., Radhika, G., Aravindhakshan, T.V. and Anilkumar, K. (2016). Expression profiling of innate immune genes in milk somatic cells during subclinical mastitis in crossbred dairy cows. *Anim. Biotechnol.* **27(4)**: 303-309.

Kumar, P., Sharma, A., Sindhu, N. and Deora, A. (2014). Acute phase proteins as indicators of inflammation in streptococcal and staphylococcal mastitis in buffaloes. *Haryana Vet.* **53(1)**: 46-49.

Lakshmi, R. and Jayavardhanan, K.K. (2016). Isolation and identification of major causing bacteria from bovine mastitis. *Int. J. Appl. Pure Sci. Agric.* **2(4)**: 45-48.

Li, F., Li, F., Chen, B., Zhou, B., Yu, P., Yu, S., Lai, W. and Xu, H. (2017). Sextuplex PCR combined with immunomagnetic separation and PMA treatment for rapid detection and specific identification of viable *Salmonella* spp., *Salmonella enterica* serovars *Paratyphi* B, *Salmonella typhimurium* and *Salmonella enteritidis* in raw meat. *Food Control.* **73**: 587-594.

Maciel-Guerra, A., Esener, N., Giebel, K., Lea, D., Green, M.J., Bradley, A.J. and Dottorini, T. (2021). Prediction of *Streptococcus uberis* clinical mastitis treatment success in dairy herds by means of mass spectrometry and machine-learning. *Sci. Rep.* **11**: 7736.

Markey, B., Leonard, F., Archambault, M., Cullinane, A. and Maguire, D. (2013). Clinical veterinary microbiology, Mosby Ltd.

Mir, A.Q., Bansal, B.K. and Gupta, D.K. (2014). Subclinical mastitis in machine milked dairy farms in Punjab: prevalence, distribution of bacteria and current antibiogram. *Vet. World.* **7(5)**: 291-294.

Mittal, D., Sharma, A., Singh, M., Rajesh and Mahajan, N.K. (2018). Antimicrobial sensitivity pattern observed in microbes associated with bovine mastitis. *Haryana Vet.* **57(2)**: 215-218.

Mohanty, N.N., Das, P., Pany, S.S., Sarangi, L.N., Ranabijuli, S. and Panda, H.K. (2013). Isolation and antibiogram of *Staphylococcus*, *Streptococcus* and *Escherichia coli* isolates from clinical and subclinical cases of bovine mastitis. *Vet. World.* **6(10)**: 739-743.

Nickerson and Stephen, C. (2009). Control of heifer mastitis: Antimicrobial treatment-An overview. *Vet. Microbiol.* **134(1-2)**: 128-135.

Pankaj, Sharma, A., Chhabra, R. and Sindhu, N. (2012). Prevalence of sub clinical mastitis in cows: its etiology and antibiogram. *Indian J. Anim. Res.* **46(4)**: 348-353.

Pankaj, Sharma, A., Chhabra, R. and Sindhu, N. (2013). Subclinical mastitis in Murrah buffaloes with special reference to prevalence, etiology and antibiogram. *Buff. Bullet.* **32**: 107-115.

Rani, L., Srinivas, M., Suresh, K. and Sreenu, M. (2008). An epidemiological study of mastitis in buffaloes. *Indian Vet. J.* **85(12)**: 1350-1351.

Sharma, L., Verma, A.K., Kumar, A., Rahat, A., Neha and Nigam, R. (2015). Incidence and pattern of antibiotic resistance of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in cattle and buffaloes. *Asian J. Anim. Sci.* **9(3)**: 100-109.

Singathia, R., Sharma, D.K., Solanki, S., Gaurav, A. and Rathore, K. (2022). Prevalence of subclinical mastitis in cattle in and around Udaipur district of Rajasthan. *Vet. Pract.* **23(2)**: 296-298.

Singh, M. (2015). Microbiological and immunological studies on buffalo subclinical mastitis. Ph.D. Thesis submitted to Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Singh, M., Sharma, A., Mittal, D. and Charaya, G. (2014). Prevalence and characterization of coagulase negative *Staphylococci* associated with buffalo mastitis. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **35(2)**: 67-72.

Singh, M., Sharma, A., Mittal, D. and Charaya, G. (2015). Prevalence of buabaline subclinical mastitis along with microbial profile and sensitivity pattern. *Haryana Vet.* **54(2)**: 157-159.

Singh, N., Singh, P. and Patel, R. K. (2016). Isolation and identification of bacterial organisms from mastitic milk. *J. Livestock Sci.* **7**: 46-48.

Solanki, S., Sharma, D.K., Singathia, R. and Devi, D. (2021). Prevalence of bovine subclinical mastitis in Sirohi district of Rajasthan. *J. Pharm. Innov.* **SP-10(7)**: 174-177.

Strommenger, B., Kettlitz, C., Werner, G. and Witte, W. (2003). Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J. Clin. Microbiol.* **41(9)**: 4089-94.

Sunagar, R., Deore, S.N., Deshpande, P.V., Rizwan, A., Sannejal, A.D., Sundareshan, S., Rawool, D.B., Barbuddhe, S.B., Jhala, M.K., Bannalikar, A.S., Mugalikar, D.M., Kumari, V.J., Dhanalakshmi, K., Reddy, Y.N., Rao, P.P., Babra, C., Tiwari, J., Mukkur, T.K., Castantino, P., Wetherall, J.D., Isloor, S. and Hegde, N.R. (2013). Differentiation of *Staphylococcus aureus* and *Staphylococcus epidermidis* by PCR for the fibrinogen binding protein gene. *J. Dairy Sci.* **96**: 2857-2865.

Tenhagen, B.A., Hansen, I., Reinecke, A. and Heuwieser, W. (2009). Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *J. Dairy Res.* **76(2)**: 179-187.

Verma, H., Shriya, R., Nishant, S., Vikas, J. and Singh, R. (2018). Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. *J. Entomol. Zool. Studies.* **6(1)**: 706-709.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In: current protocols in molecular biology. Vol. 1, F. M. Ausubal, R. Brent, R.L., Kirston, D.D., Moore, J.G. Seidman, J.A., Smith, and K. Struhe (eds.) John Wiley and Sons, New York, pp. 241-242.