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Research Article

MICROBIAL QUALITY OF MILK FLOW DISORDERS IN HAND MILKING DAIRY COWS

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ABSTRACT
Thirty two animals were included milk flow disorder (MFD) evaluation. Out of 32 animals 23 (71.86) were MFD affected animals. The mean values of SCC were more in MFD affected with mastitis quarters than in MFD affected without mastitis quarters. In MFD affected quarters bacterial isolates of *Staphylococcus aureus* (4.69), *Streptococcus agalactiae* (2.34), *Bacillus cereus* (1.56), *E.coli* (0.78), mixed infection (8.59) and culture negative (3.91) per cent were observed. Out of 23 MFD affected 34.3 (n=11) per cent of animals had subclinical mastitis, 28.12 (n=9) per cent of animals had clinical mastitis and 9.4 (n=3) per cent animals did not have mastitis. Therefore, milk flow disorder affected cows are more prone for subclinical mastitis than clinical mastitis.

Keywords: *Microbial Quality, Milk Flow Disorder, Dairy Cow*

INTRODUCTION
Subclinical mastitis foremost disease encountered in field level and causes great economic loss to the farmers. Milk flow disorders cause economic losses because it not only affects the quality of milk but also the milk yield Querengässer *et al.,* (2002a). Such economic loss warrants different strategies in the selection and removal of animals which are susceptible to disease so that the occurrence of mastitis can be minimized. Less data is available on parameters like association of milk flow disorders with milk yield, teat morphometry, ultrasonographic variables of teat, Somatic Cell Count and organisms involved. Therefore, the present study is planned to evaluate the status of mastitis in cows with milk flow disorders.

MATERIALS AND METHODS
The research was conducted on animals, which were presented to the Large Animal Medical Outpatient Unit of the Madras Veterinary College Teaching Hospital, University Research Farms and Private dairy farm in and around Chennai.

Cases were screened for milk flow disorders based on the history of milk flow, physical examination and ultrasonography of teat by water bath technique using linear probe 7.5 MHz to 12.5 MHz. Approximately 10 ml of fore milk was collected into sterile (polythene) culture tubes from all the four teats.

Tests included physical properties of milk, Californian Mastitis Test (CMT), Somatic cell count (SCC) was performed by DeLaval Cell Counter ^{R} (Delaval international, USA) as per the manufacturer's instructions.

Bacterial Culture studies were conducted as per the standard protocol by using specific media for each bacteria (Agar medium O (Baird-parker agar), β-S*treptococcus* selective agar base, *Bacillus cereus* agar base, EMB broth (Hi Media Laboratories Ltd., Mumbai, India) and the biochemical tests were done by using organism specific biochemical test kit (*Staphylococcus spp, Streptococcus spp, E.coli* and *Bacillus spp*) for characterization of individual bacteria as per the manufacturer's instructions.
The DNA isolated from milk sample and colony was amplified by PCR using universal 16s rRNA primers and specific primers (Sigma, USA) and the purified PCR products were sequenced using applied biosystem kit. The homology of the PCR products was checked by BLAST analysis with the sequences already available in the NCBI, Gen bank.

RESULTS AND DISCUSSION

In the present study Milk flow disorder affected animals were 23 (71.86 per cent) and without Milk flow disorders were 9 (28.12 per cent) out of 32 animals. Out of 23 MFD affected 34.3 (n=11) per cent of animals had subclinical mastitis, 28.12 (n=9) per cent of animals had clinical mastitis and 9.4 (n=3) per cent animals did not have mastitis. Therefore, milk flow disorder affected cows are more prone for subclinical mastitis than clinical mastitis. Bhatt *et al.,* (2010) in his study reported that out of 20 buffaloes with milk flow disorder, 17 cases (85 per cent) had subclinical mastitis, 3 cases (15 per cent) had clinical mastitis Milk flow disorders increased the risk of subclinical and clinical mastitis (Agger and Willederg, 1986).

Out of 128 quarters examined for MFD with mastitis (n= 20), MFD without mastitis (n= 8) and mastitis without MFD (n= 76) and healthy quarter (n= 24)16, 6, 59 and 19 per cent were observed. In the present study the mean values of SCC were more in MFD affected with mastitis quarters than in MFD affected without mastitis quarters and also the mean values of SCC were more in MFD unaffected with mastitis quarters than in MFD unaffected without mastitis quarters.

Out of 128 teats examined, 28 teats had milk flow disorders. In MFD affected quarters bacterial isolates of *Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus, E.coli,* mixed infection and culture negative 4.69, 2.34, 1.56, 0.78, 8.59 and 3.91 per cent were observed respectively.

Querengässer *et al.,* (2002 b) found that the Somatic Cell Count (SCC), the odds of detecting pathogens, or the odds of mastitis were higher in milk from teats with milk flow disorders than in the milk from teats without milk flow disorders.

In MFD unaffected quarters bacterial isolates of *Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus, E.coli,* mixed infection and culture negative 17.19 , 0.78 , 5.47, 1.56, 35.93 per cent and 17.97 per cent were observed respectively.

Amin *et al.,* (2011) used PCR assays for detecting major pathogens causing mastitis. They have used specific primers for amplification of DNA from *Staphylococcus aureus* (17.9%), *Streptococcus agalactiae* (16.1%) and *Escherichia coli* (35.7%) in case of clinical cases, while in case of sub-clinical cases *Staphylococcus aureus* (33.8%), *Streptococcus agalactiae* (25.0%) and *Escherichia coli* (11.8%) were amplified. In the present study *Staphylococcus aureus* (33.33 %), *Streptococcus agalactiae* (22.22 %) and *Escherichia coli* (11.11 %) in case of clinical cases, while in case of sub-clinical cases *Staphylococcus aureus* (54.45 %), *Streptococcus agalactiae* (27.27 %) and *Escherichia coli* (18.18 %) were amplified by using specific primers for amplification of DNA.

In this study mixed infection bacteria were also detected from samples from clinical and subclinical mastitis with MFD teats (*Staphylococcus aureus* and *Streptococcus agalaciae*). This concurred with the findings of Amin *et al.,* (2011).

They also reported that mixed bacterial infection from milk samples collected from clinical mastitis [*Staphylococcus aureus* and *Escherichia coli* (7.1%); *Staphylococcus aureus and Streptococcus agalactiae* (7.1%) and *Escherichia coli* and *Streptococcus agalactiae* (5.4%)] as well as sub-clinical mastitis 8.8, 4.4 and 4.4% respectively. 1.8 and 2.9 per cent samples were negative in samples from clinical and sub-clinical mastitis cows, respectively.

Specific PCR primers were used for amplification of DNA fragments obtained from *Staphylococcus aureus, Streptococcus agalactiae, E. coli and Bacillus cereus* (*gyr*B gene). The size of the PCR products was 1318 bp, 405 bp, 232 bp and 374 bp.

The sequences of the primers used and PCR product size are presented in Table 1. The agarose gels with the PCR products are presented in **Plates 1a, 1b and 1c.** The gel eluted PCR products were sequenced and the sequences showed 90-98 per cent homology with the sequences available in the NCBI Gen Bank database using BLAST sequence- homology search.

Table 1: Specific primers used for identification of bacteria from milk sample and isolated colony by PCR

Primers		Sequence (5' – 3')	Size of the product
Staphylococcus aureus	Amin *et al.*, (2011)	GGACGACATTAGACGAATCA CGGGCACCTATTTTCTATCT	1318 bp
Streptococcus agalactiae	Amin *et al.*, (2011)	CGCTGAGGTTTGGTGTTTACA CACTCCTACCAACGTTCTTC	405 bp
E.coli	Amin *et al.*, (2011)	ATCAACCGAGATTCCCCCAGT TCACTATCGAGTCAGGAG	232 bp
Bacillus cereus	Manzano *et al.*, (2003)	GTTTCTGGTGGTTTACATGG TTTTGAGCGATTTAAATGC	374 bp

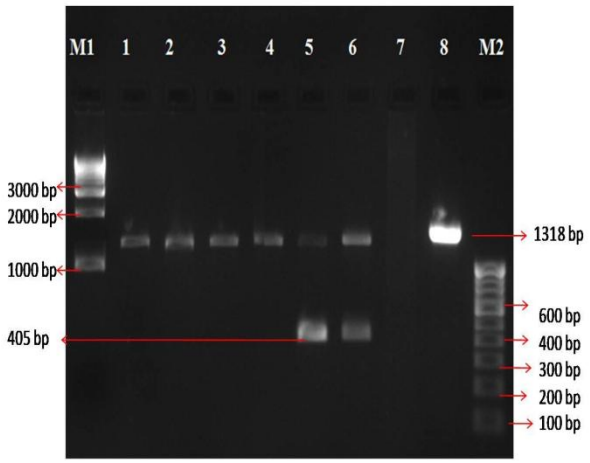

Plate 1a: 1.2% agarose gel electrophoresis of PCR products: Lane 1-4 and 8*: Staphylococcus aureus* (1318 bp); Lane 5 and 6: *Staphylococcus aureus* and *Streptococcus agalactiae* (405 bp) respectively; Lane M1and M2: 1 Kb and 100 bp ladder

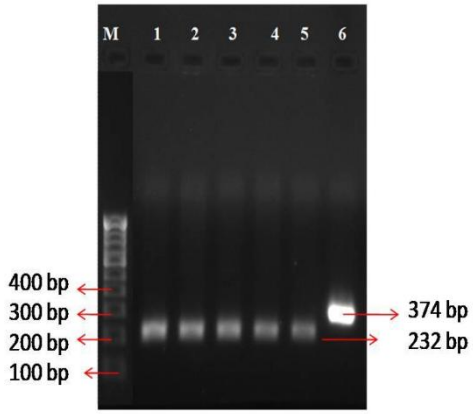

Plate 1b: 1.2% agarose gel electrophoresis of PCR products. Lane 1-5: *E.coli* (232 bp); Lane 6: *Bacillus cereus* (374 bp); Lane M: 100 bp ladder

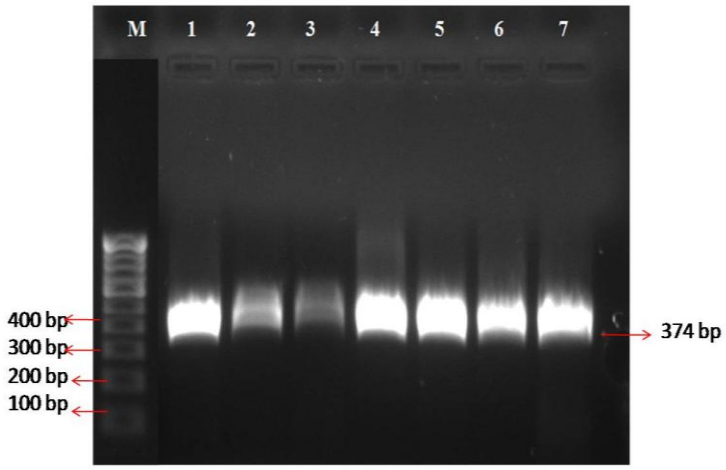

Plate 1c: 1.2 % agarose gel electrophoresis of PCR products Lane 1-7: *Bacillus cereus* (374 bp); Lane M: 100 bp ladder

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