Detection of pathogen in milk collected from an infected *Karan Fries* cow

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Abstract

Mastitis is a multi-etiologic disease of the mammary gland characterized mainly by reduction in milk production and milk quality due to intramammary infection by pathogenic bacteria. Nearly 83% of lactating dairy cows in India are infected with mastitis with various degree of inflammation. In this study, we determined differential count of somatic cell count, bacterial load and presence of *S. aureus* in milk collected from healthy, sub-clinical (SCM) and clinical mastitis (CM) *Karan Fries* cows. On the basis of SCC and CMT score milk was categorized into Healthy, SCM and CM. The SCM and CM milk samples were positive for *S. aureus* on MSA and Baird Parker Agar media while healthy milk samples were negative for *S. aureus.*

Keywords: Intramammary infection, Sub-Clinical Mastitis, Clinical Mastitis, Somatic cell count, California mastitis test, Bacteriological.

Introduction

Milk is largely made up of water, within which a wide range of nutrients including vitamins, proteins, fats and carbohydrates are suspended [1]. These rich nutritional contents and the production and processing procedures in commercial milk production render it susceptible to contamination by a host of pathogenic microbes that could cause diseases in humans. Therefore, milk is known to be an efficient vehicle for transmission of disease-causing agents to humans [2] and represents the serious health threat to consumers worldwide. The microbial growth has negative impact on product productivity and quality [3].

Mastitis remains one of the most prevalent reason of bacterial load in milk and economically detrimental diseases in dairy farms worldwide [4].

The inflammation of the mammary gland predominantly caused by coagulase-negative *Staphylococci*, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus aureus (S. aureus),* and *Escherichia coli (E. coli)* [5]. Therefore, the purpose of present study to identify the pathogenic bacteria in milk collected from healthy, sub-clinical infected and clinical infected milk. The bacteriological identification in milk allows to detect the causative agent of disease in herd and helps to provide preventive treatment at accurate time.

Material and Method

Milk collection

Milk sample were collected on quarter basis from 25 *Karan Fries* (quarter-100) cows from Cattle from local area. The animal selected for milk collection were from various lactation period namely such as early, mid and late stages (50-250).

Determination of Somatic cell count

The somatic cell count was determined by using Eko-milk Scan (Eon Trading LLC, USA) within two hour of milk collection. The milk (5ml) and detergent solution (10ml) was poured in flask mounted on sample mixture in 1:2 ratio and allowed to mix thoroughly by rotational mixing using 10 cycles of swings. After mixing sample was dripped off as a gel and somatic count were displayed on screen.

California Mastitis test:

California Mastitis Test (CMT) was performed as prescribed by Schalm and Noorlander (1957) with minor modifications.[6] 1ml of milk from each quarter was poured into four well paddle. Equal amount (1ml) of CMT reagent was added into each well. The gentle shaking was done for proper mixing and observe the gel/flakes formation in each well. The wells were scored 0 to 3 on the basis of gel formation.

Determination of Bacterial Count

The Standard Plate count method was used for detection of bacterial load in milk collected from healthy, subclinical and clinical mastitis animals. 1ml of milk from each category was added into 5ml of LB Broth at 37^0 C for overnight enrichment.

Then enriched bacteria in milk samples were diluted (10^{-1} to 10^{-9}) using peptone water and plated on LB media and incubate at 37^0C for 24 hrs.

Detection of *Staphylococcus Aureus* in milk

Mannitol Salt Agar for the detection of *Staphylococcus aureus*

A loopful enriched milk from each group was plated on solidified MSA media and incubated at 37^0C for 48 hrs. The plates were observed for media color change and identify the presence of *S.aureus*.

Detection of *Staphylococcus aureus* on Baird Parker agar

1ml of enriched milk from each group was plated on solidified media and incubated at 37^0C for 48 hrs. The plates were observed for black/grey color selective colonies of *S.aureus*.

Results and Discussion

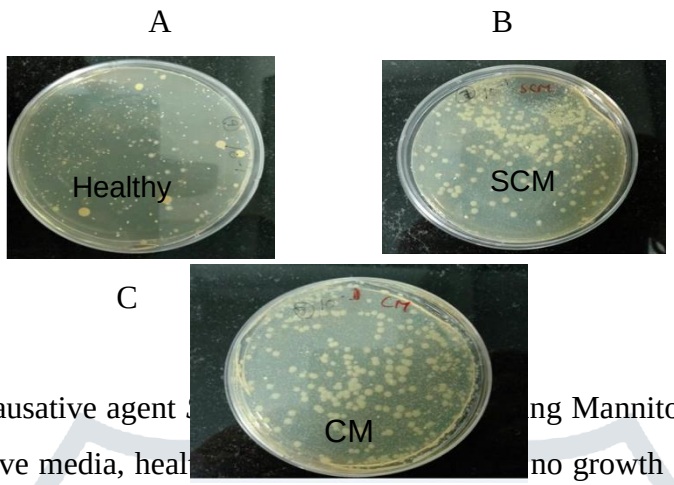
The SCC is considered as important breast health index in cows and primary indicator of infection. The significant increase of SCC in milk associated poor quality of milk due to low level of lactose, protein, fat and relatively high level of milk pH, sodium and chloride content (electrical conductivity).
The milk samples were categorized by SCC threshold for this study was: Healthy ($7x10^5$- $1x10^6$ cells/ml), SCM ($2\text{-}3.5x10^6$ cells/ml) and CM ($13\text{-}15x10^6$ cells/ml).

Out of 100 samples, 35% samples had no gel formation and scored 0 was indicates the non-infective samples. In 24% samples minor gel formation observed and scored as 1&2 showing sub-acute infection. But in 37% samples solid gel formation was observed and scored as 3&4 which confirmed the severe infection in udder. The correlation of SCC and CMT shown in Table 1.

Health Status	**SCC**	**CMT**
Healthy	70-1 lac	0
SCM	2-5 lac	+ ++
CM	13-15 lac	+++

The enhanced somatic cell count was observed in lipopolysaccharide and lipoteichoic-acid-induced mastitis as compare to healthy bovine milk [7]. The effect of increased SCC on milk composition such protein, lactose, fat and SNF was evaluated by Reis et al (2013). This study showed that IMI has negatively effects on milk composition, although it has been shown that the degree of changes depends on the inflammatory response, the severity and amount of affected tissue in the mammary gland, and bacterial pathogenicity [8].

The healthy, SCM and CM milk were showing differential bacterial load. The bacterial count in heathy milk was less than 30 cfu/ml and for subclinical and clinical milk it was 30-300 cfu/ml and > 300 cfu/ml respectively shown in Fig.



The presence of mastitis causative agent *S* ng Mannitol Salt Agar and Baird Parker agar media. In both selective media, heal no growth of *S.aurus* but SCM and CM were s

Figure 1: Detection of Bacterial Load Milk Collected from (A) Healthy (B) Sub-clinical Mastitis (SCM) (C) Clinical Mastitis (CM)

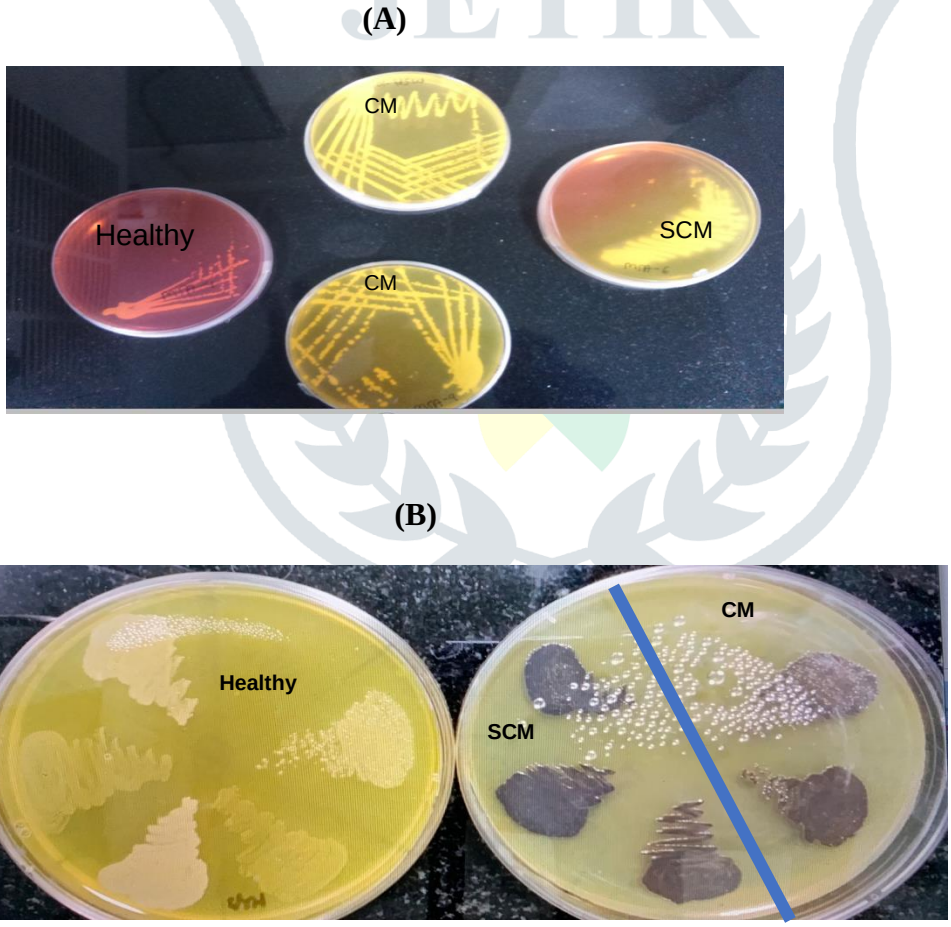


Figure 2: Selective Detection of S.aures in Healthy, Sub-clinical Mastitis (SCM) and Clinical Mastitis (CM) milk on (A) Mannitol Salt Agar (B) Baird Parker agar media

Harjanti et al (2018) reported that the bacteriological examination of milk samples revealed that Streptococcus was predominant species (73.3%) and the coagulase negative Staphylococcus species was identified at the least bacteria (26.7%) in analyzed samples [9].

Another study reported that 87staphylococcal isolates were found in milk collected from sub clinical mastitis buffalo, bovine ovine and caprine. Total 58 isolates were positive for mannitol fermentation and remaining 30 isolates produced agglutination with the Staphytech plus kit leads to acetoin production and were classified as *S. aureus* [10].

Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) was found in bovine mastitis. The *mec*A gene present in MRSA bacteria confers resistance to almost all *β*-lactam antibiotics which are the most frequent drugs used in bovine mastitis therapy [11].

Conclusion

In this present study, IMI resulted in increased SCC which was associated with reduced milk production and poor quality of milk. The enhanced SCC reflects the elicited immune response due to invasion of pathogen. More studies are necessary to better understand the variation of mastitis pathogens according to the age, parity, lactation stage and infection stage of the cow.

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