ISSN: 2278-294x (Print), ISSN: 2278- 2958 (Online)

Indian Journal of Drugs and Diseases

Study on prevalence of bovine mastitis on dairy cows in and around Coimbatore district, Tamilnadu, South India

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Abstract

In this study, 250 lactating cows are tested using standard procedures. Prevalence of mastitis at cow level was 66.0% (165/250), out of which 26.06 % (43/165) and 75.30 % (122/165) were clinical and subclinical, respectively. The quarter level of prevalence was 66.0% (660/1000); from this the clinical and subclinical forms were 26.06% (172/660) and 73.93% (488/660), respectively. The data revealed that *Staphylococcus aureus* was the commonest organism in mastitis cases, being implicated in 70.30% cases, and *Streptococcus agalactiae* (5.45%) continues to be a prevalent pathogen followed by *S.epidermis* (4.24%), *Proteus* sp (3.63%), *Bacillus* sp (2.42%), *Streptococcus uberis* (2.42%), *E.coli* (1.81%) and *Micrococcus* sp (8.48%). In addition, *Str. agalactiae* and *S. aureus* were more frequently associated with clinical mastitis than sub-clinical case, while the infection rates of other bacteria were similar. In conclusion, this study revealed the importance of mastitis and associated bacterial pathogen in the study area.

Key words: Antibiotics, mastitis, pathogens, udder

Introduction

India stands for first in milk production in the World. Infection of the cow's udder (bovine mastitis) has remained one of the major constraints in growth of dairy industry in India and abroad. Amongst cattle diseases, bovine mastitis is a serious problem, which affects the basic income of the farmers depleting their dairy sources. Worldwide mastitis is associated with economic losses of $ 35 billion annually. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases (Muhamed Mubarack *et al.,* 2011).

Mastitis is an important disease of dairy animals. It is an inflammation of the mammary gland (udder) that causes physical and chemical changes in milk and leads to pathological condition of the glandular tissue. It is generally associated with poor hygienic and husbandry practices. This disease can have an infectious or noninfectious etiology, and the infectious pathogeny is the most important ones that frequently due to infection by one and/or the other pathogens, such as bacteria, viruses, mycoplasma, yeasts and algae (DaRong Cheng *et al.,* 2010). Majority of microorganisms that are responsible for mastitis and spoilage of milk could be *Staphylococcus aureus*, *Streptococcus agalactiae Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis*, coliforms (*Escherichia coli*, *Klebsiella* species and *Enterobacter aerogenes), Serratia, Pseudomonas, Proteus* species, environmental *Streptococci, Enterobacte*r species (Bedada & Hiko, 2011). The aim of this research was to characterize the bacteria isolates from bovine mastitis in and around Coimbatore District and to investigate into the possible infection types within problem herds.

Materials and methods

Collection of samples

250 lactating cows were examined from 87 dairy herds in different smallholder farms in

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and around Coimbatore District, Tamilnadu, SouthIndia during May 2009 - April 2010. Prior to sampling, teat ends were swabbed with 70% ethylalcohol. The initial milk stripped from each udder (one udder each cow) was discarded and the next 10 ml were collected in a sterile container. Samples were chilled to 4°C and transported to the laboratory (Boynukara *et al*., 2008; Ericsson Unnerstad *et al*., 2009; White *et al*., 1999). The more detailed information was illustrated in Table 1.

Table 1.Prevalence of clinical and subclinical mastitis in dairy cows

	Considered factor	Total examined (N = 250)
	Types of Mastitis	Mastitis positive No.(%)
Cow	Clinical	43
	Subclinical	122
Udder	Clinical	172
	Subclinical	488

Bacterial pre-culturing

Transfer each raw milk (500µl) into separate tubes containing 5 ml of nutrient broth (10 g tryptone, 5g beef extract, 10g NaCl, 0.5g K_2HPO_3, H_2O to 1 liter, pH 7.4) with 10% heat - inactivated fetal calves serum (FCS) and grow the liquid cultures with vigorous agitation at 37°C for 18 h.

Isolation and identification of Staphylococcus

From each pre - cultured sample, a loop full of bacteria suspension was streaked on Mannitol Salt Agar (MSA) that was widely used to cultivate *Staphylococcus* from clinical specimens and then incubated at 37°C for 48 h under aerobic conditions (Boynukara *et al.,* 2008; Cenci-Goga *et al.,* 2003). Ten presumptive colonies were randomly selected and transferred to individual plate of nutrient agar to make pure culture of bacteria isolates. Following incubation for 24 h under the same condition, a single colony of bacteria was streaked on MSA plate. Yellow colored colonies were mannitol - positive and suspected as *S. aureus* or *S. saprophyticus*, while red colored colonies were mannitol - negative and suspected as *S. epidermids* or *S. saprophyticus* (Boynukara *et al*., 2008). Subsequently, gram staining, pigment producing, maltose fermentation test, alkaline phosphatase test, catalase test, polymyxin B susceptibility test, coagulate test using fresh rabbit plasma (tube method) and DNase test (determine DNase production and activity) were used for the presumptive identification of all isolates (Gundogan *et al*., 2006; Monsen *et al*., 1998).

Isolation and identification of Streptococcus

From each pre-cultured sample, a loop-full of bacteria suspension was streaked on Colistin- oxolinic acid blood agar (COBA) plate(Guangzhou Huikang Biotech Co., Ltd. China) and then incubated at 37°C for 48 h under aerobic conditions. Ten pinpoint and dewdrop - as if colonies were randomly selected and transferred to individual plate of nutrient agar with 10% heat - inactivated FCS to make pure culture of bacteria isolates. Following incubation for 24h under the same condition, gram staining and conventional biochemical tests, including catalase assay, esculin hydrolysis test, sodium hippurate hydrolysis test, sugar (lactose, synanthrin, mannitol and sorbitol) fermentation test (Cao, 1991; Ericsson *et al.,* 2009), characterized each presumptive bacteria isolate.

Results and discussion

Mastitis is the most important disease in dairy milk production worldwide, and it is notoriously difficult to estimate the losses associated with clinical and sub - clinical mastitis, which arise from the costs of treatment, culling, death and decreased milk production and constituent quality Classically, mastitis pathogens have been classified as either “contagious” or environmental. In essence, the contagious pathogens can be considered as organisms adapted to survive within the mammary gland, and can establish infections to trigger inflammatory response, which are typically manifest as an elevation in the somatic cell count of milk from the affected quarter (Bradley, 2002).

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Table 3.Frequency of various types of bacteria isolated from clinical and subclinical cases			
Types of Bacterial Isolates	Mastitis Positive (N = 165)	Types of Mastitis	
		Clinical (n = 43)	Subclinical (n = 122)
S.aureus	116 (70.30)	26 (60.46)	90 (73.77)
S.epidermidis	7 (4.24)	- (0.00)	7 (5.73)
Bacillus sp	4 (2.42)	1 (2.32)	3 (2.45)
Proteus sp.	6 (3.63)	3 (6.97)	3 (2.45)
E.coli	3 (1.81)	1 (2.32)	2 (1.63)
Str.uberis	4 (2.42)	1 (2.32)	3 (2.45)
Str. agalactiae	9 (5.45)	- (0.00)	9 (7.37)
Micrococcus sp.	14(8.48)	3 (6.97)	11 (9.01)

Prevalence of clinical mastitis

In this study, 250 cows were examined of which 165 cows (43 clinical and 122 subclinical cases) and the data was presented in Table 1. It revealed that *S.aureus* was the commonest organism in mastitis cases, being implicated in 70.30% cases. *S.aureus* (70.30%) continues to be a prevalent pathogen followed by *Streptococcus agalactiae* (5.45%), *S.epidermis* (4.24%), *Proteus* sp (3.63%), *Bacillus* sp (2.42%), *Streptococcus uberis* (2.42%) and *E.coli* (1.81%).*Micrococcus sp* (8.48%) previously considered as environmental organisms live in the environment and contaminates the teats, was also detected in the problem cows (Table 3). *Staphylococcus* and *Streptococcus*, considered being the major contagious pathogens of bovine mastitis, frequently combined and mixed infection with *E. coli*.

Table 2. Comparison of infection type between clinical and sub-clinical bovine mastitis		
Mastitis Pathogens	Types of Mastitis	
	Clinical	Subclinical
Staphylococcus aureus	+	+
Staphylococcus epidermidis	+	+
Bacillus sp	+	-
Proteus sp.	+	-
E.coli	+	+
Streptococcus uberis	+	+
Streptococcus agalactiae	+	+
Micrococcus sp.	+	-

Different bacterial infection between clinical and sub - clinical mastitis

In summary, all the eight species of bacteria (*S. aureus*, *S. epidermids, Bacillus* sp., *Proteus* sp., *E. coli*, *Str. uberis*, *Str. agalactiae*, and *Micrococcus* sp.) could be identified in both clinical and sub - clinical bovine mastitis (Table 2).

At the same time, *S. aureus* and *Micrococcus* sp and were more frequently found in clinical mastitis than sub-clinical case, while the infection rates of *E. coli*, *Str. agalactiae* and *S. epidermids* were similar in statistics between clinical and sub-clinical bovine mastitis.

Staphylococcus aureus was the major mastitis-inducing pathogens detected in this study (Table 3). This is similar to the findings of Kewler *et al* (1992) who reported *Staphylococcus aureus* to be the most common cause of bovine mastitis. The isolation of *staphylococcus aureus* is of public health significance since it is a commonly recovered pathogen in outbreaks of food poising due to milk and milk product (Junaidu *et al*., 2011).

The statistics of this research not only confirmed the prevalence of the five vast major species of bacteria (*E. coli*, *Str. uberis*, *S. aureus*, *Str. dysgalactiae* and *Str. agalactia.*) in part region of China, but also found *S. epidermids* and *S. saprophyticus*, previously considered as naught pathogenic bacteria, were existed in the diseased mammary gland of the problem cows (DaRong Cheng *et al.,* 2010).

In adequate hygienic condition of dairy environment, poor milking procedure, poor animal health service and lack of proper attention to health of the mammary gland were important for the high prevalence of mastitis factors in the study farms. Adequate housing with proper sanitation and regular screening for early detection and treatment, follow up of chronic case, culling of older cows with repeated attacks, avoiding consecutive milking and susceptibility testing of the mastitis pathogens before treatment are recommended to alleviate the problem.

Acknowledgement

The first and second authors are grateful to University Grant Commission (UGC) for the financial support given to the present study under the Major Research Project programme

ISSN: 2278-294x (Print), ISSN: 2278- 2958 (Online)

entitled "A Study of Ethno-veterinary Medicinal Plants and *in-vitro* antimicrobial activities against Bovine Mastitis isolated bacterial pathogens"[Sanction No. F. No. 35-121/2008 (SR) dt.20 March 2009]. The authors are thankful to the management of RVS Educational Trust for their encouragement and support.

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