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Isolation and identification of major causing bacteria from bovinemastitis

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Abstract

The present study was conducted to isolate and identify the major causing bacteria from mastitis milk sample collected from Kerala veterinaryUniversity Livestock Farm, Mannuthy. A total of fifty milk samples were collected from mastitis affected animals and microbiological testing of raw milk was done. Upon microbiological testing the major causing bacteria Staphylococcus aureusand Escherichia coli, organisms were identified. A total of 31 isolates were identified in milk samples asmajor pathogen, of which 18 (36%) affected with Staphylococcusaurues followed by 13 (27%) with E. coliorganisms. Microbiological testing is necessary for identification of cause of mastitis and adaptation of control measure to get rid of infection.

Key Words: Mastitis, bacteria, culture, biochemical test, S. aureusandEscherichia coli

I. Introduction

Bovine mastitis is the one of the most prevalent costly disease for dairy farmers and industry, resulting in a great deal of economic losses, mostly because of reduction of milk yield, decreased milk quality, higher production and medication costs, loss of milking days, reduced milk price, increased labour (Seegers*et al.,*2003 ; Cremonesi*et al.,* 2006). Mastitis is also affecting the quality of dairy products, and reducing their shelf life.Most estimates views that on the average and affected quarter suffered a 30 % reduction in productivity and an affected cow estimated to lose 15 %t of its production for lactation (Prasad*,* 2001).

There are two forms of mastitis prevalent in terms of level of severity; clinical and subclinical. Clinical form of mastitis shows visible symptoms whereas subclinical form does not show any visible symptoms. Clinical mastitis is threatening to a farmer in a dairy herd and treatment is given immediately to control it. But subclinical mastitis, which cannot be identified without a laboratory or field test, mostly remains unnoticed by the farmer.

In India incidence of subclinical form mastitis was found to be more (varying from 10-50% in cows and 5-20% in buffaloes) when compared to clinical mastitis (1-10%). Annual losses in the dairy industry due to mastitis was almost 526 million dollars (2.37 thousand crore Rupees) in India, in which subclinical mastitis are subjected for approximately 70% of these losses (Varshney and Naresh, 2004).

Although mastitis is caused byvarious bacteria, viruses (Wellenberg*et al*., 2002), and fungi (Farnsworth, 1977) the most common cause are gram-positive and gram negative bacteria (Zecconi*et al*., 2005). The immune response of the mammary gland varies towards different bacterial infection (Lee *et al.*, 2006). The two most common bacterial cause of mastitis are *Escherichia coli* (Hogan and

Smith, 2003) and *Staphylococcus aureus* (Zecconi*et al*., 2006). Therefore present study was conducted to isolate the major causing bacteria from mastitis milk samples.

II. Materials and Methods

2.1 Collection of milk samples

Milk samples were collected from Kerala veterinaryUniversity Livestock Farm, Mannuthy. Fifty milk samples were collected from cows aseptically using sterile vials and stored at 4°C until processed.

2.2 Processing of the samples

Milk samples were mixed well and two or three loopful of milk was streaked on Mueller Hinton (MH) agar (Gram-postive bacteria) and MacConkey's agar plates (Gram-negative bacteria) and incubated at 37ºC for 24-48 hrs. The cultured organisms were subjected for bacteriological analysis. A minimum of five colonies of the same type was recorded as causative agent and growth more than one type of colonies was determined as mixed growth. Colonies were subjected for gram staining and biochemical test for the identification of causative agent.

2.3 Gram staining

The smear was prepared from positive culture and flooded with crystal violet solution for two minutes. Then the slide was washed with distilled water and gram's Iodine was applied for one minute. After that 95% alcohol was applied until the colour runs off. Finally dilute carbholfuchin was applied for about one minute. Then the slide was washed with distilled water and examined under oil immersion. Identified gram positive and negative bacteria are subjected for biochemical test.

2.4 Biochemical tests for *Staphylococcus aureus* and *E. coli*

For identification of causal organism identified gram positive and negative samples were subjected for following biochemical test. Identification of causal organism based on biochemical test is summarized in Table 1.

- ***Catalase test:***A loopful of test culture on MH agar was mixed with 2-3 drops of 3 percent hydrogen peroxide on a clean glass slide and examined for the release of nascent oxygen in the form of gas bubbles. A positive reaction was indicated by the effervescence of oxygen within 1-2 minutes.
- ***Oxidase activity:***Surface growth of a test culture on MH agar was smeared with the glass rod on the filter paper strip impregnated with oxidase reagent. A positive reaction was indicated by the appearance of a dark purple colour within 30 sec.
- ***Citrate utilization test:***The culture under test was streaked onto Simmon's citrate slants and incubated at 37ºC for 96 hrs. A positive reaction was indicated by appearance of blue colour and growth on the streak line.
- ***Indole test:***The culture under test was inoculated into Luria broth and incubated at 37ºC for 48 hrs. Kovac's reagent 0.5 ml was added and shaken gently. A positive reaction was indicated by the appearance of red top layer over the medium.
- ***Methyl red test:***The test culture was inoculated into the methyl red medium and incubated at 37ºC for 2-5 days. Five drops of the methyl red reagent was added and mixed well. A positive reaction was indicated by the appearance of a bright red colour immediately after mixing.

Table 1. Biochemical test for identification of *S. aureus and E. coli*

Test name	*S. aureus*	*E. coli*
Catalase	+	_
Oxidase	_	_
Simmons's citrate	+	-
Indole test	-	+
Methyl red test	+	+

III. Results and Discussion

A total of 50 mastitis milk samples were collected. For microbial culture, all 50 samples were inoculated in MH medium for identification *Staphyloccousspp* and Macconkey's*medium* for identification C*oliform*. The culture positive organisms were taken and gram stained. Based on morphology the bacterial cultures were differentiated into gram positive (*Staphyloccous spp*.) and gram negative (coliforms) samples. Out of 50 samples, 28 samples found to be gram positive and 19 samples found to be positive for gram negative.

Gram positive and negative samples were further examined for biochemical test to differentiate into *S.aureus* and *E. coli* caused mastitis. Positive for catalase, methyl red, citrate test and negative for oxidase, indole test indicates *S.aureus*caused mastitis. Out of 28gram positive samples tested, 18(36%) samples were found to be positive for *S.aureus*. Similarly samples of gram negative coliforms were further subjected for biochemical test to identify the *E.coli*caused mastitis samples. *P*ositive for methyl red and indole testindicates *E.coli*caused mastitis. Out of 19 gram negative samples tested, 13 (27%) samples were found to be positive for *E.coli.*From the screening result it was found that*S.aureus* caused mastitis relatively higher in proportion than *E.coli.* The present finding is in supported by Sumathi*et al.* (2008) where they tested 60 milksamples out of which 40% *Staphylococcus*, 20% *Escherichia coli,* and remaining percentage are other bacteria were isolated from milk samples.

Mastitis caused by *staphylococcsaureus* is most prevalent contagious pathogen of bovine mastitis with a characteristic pathogenicity and poses serious problems to the dairy industry. Around 19 to 40% of cows are infected with this organism and that infected cows produce less milk as compared to non-infected cows.Staphylococcus comes from unhygienic practices and via milkers hand. *E. coli*origin takes place from contaminated environment and poor hygienic condition and it causes infection in udder viagaining entry through teat canal (Mallikarjunaswamy and Murthy, 1997). For control of mastitis hygienic practices of farm and environment should be adopted. Control programs are focused on detection of mastitis, identification of the causative agent(s) and prevention of transmission by removing the source of the agent (milk contaminated fomites, bedding, persistently infected cows, etc.). Knowledge of mammary anatomy and physiology, mammary defense mechanism, microbial habitats, microbial virulence factors, milking machine function, and antibiotics or germicides is important in achieving effective mastitis control.

IV. Conclusion

Microbiological testing is necessary for identification of etiological agent of mastitis and to develop the antimicrobial therapy for particular organism and adaptation of control measure to get rid of infection.

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