Isolation and identification of bacterial organisms from mastitic milk

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Abstract

The present study was conducted to isolate and identify mastitis causing organism in raw milk collected from different area of Rewa (M.P.). Seventy milk samples were collected from different localities and microbiological testing of raw milk was done. Up on microbiological testing various organism viz. *Staphylococcus, Streptococcus, E. coli, and Kebsiella* organisms were identified. A total of 24 isolates were identified in milk samples including major pathogen 10 (41.66%) *Staphylococcus,* followed by 7 (29.16%) *Streptococcus,* 4 (16.66%) *E. coli* and 3 (12.5%) *Klebsiella* organisms. Microbiological testing is necessary for identification of cause of mastitis and adaptation of control measure to get rid of infection.

Keywords: Mastitis; Microbiological testing; *Staphylococcus; Streptococcus; E. coli.*

Introduction

Mastitis is a major economic disease of dairy cattle which affect the production and livelihood of farmer. It was estimated that mastitis alone causes 70 % production losses in the farm (Sumathi et al., 2008). An inflammation of the mammary gland is called mastitis (Suojala *et al.,* 2011). There are abnormalities in milk like increased somatic cells, especially leukocytes and pathological changes in the mammary tissue, and inflammatory signs mainly heat, redness, swelling, hardness and pain seen in case of mastitis (Ranjan *et al.,* 2011). Mastitis occurs in clinical and subclinical forms. In clinical form there are detectable changes in mammary tissue and subclinical mastitis is recognized mainly by indirect detection: the somatic cell count in milk (Hokmabad *et al.,* 2011).

There are various microorganisms that have been described as causative agents of bovine mastitis (Watts, 1988). *Staphylococcus* has been identified as a causative agent of both clinical (Gruet *et al.,* 2001) and subclinical mastitis (Awale *et al.,* 2012). *Escherichia coli, Klebsiella spp.* are causative agents of clinical mastitis, while *S. agalactiae* are associated with subclinical mastitis (Bradley, 2002, Barkema *et al.,* 2009). Therefore present study was conducted to isolate the mastitis causing organism in milk.

Materials and methods

Collection of milk samples

Milk samples were collected from the cows of different villages in the vicinity of College of Veterinary Science and Animal Husbandry, Rewa (M.P.) where the mastitis was a frequent problem. Seventy milk samples were collected from cows aseptically using sterile vials and stored at 4°C until processed.

Processing of the samples

Milk samples were centrifuged at 2000 rpm for 15 minutes, the supernatant discarded and the sediment re-suspended in 0.05 ml of saline (Zecconi *et al.,* 1997). Diluted milk samples were directly inoculated in the nutrient broth aseptically, after inoculation samples were incubated at 37°C for 24 hour. After Gram staining procedure according to morphological analysis various selective media were used to isolate the microorganism. All media used in the study were purchased from HiMedia, Mumbai and prepared in laboratory as per standard procedure (Cruickshank *et al.,* 1975). For isolation of Gram negative organism Mac-conkey agar was used, and after growth on mac-conkey, EMB used as selective media. Mannitol salt agar and Edward media were used for the isolation of *Staphylococcus* and *Streptococcus* respectively.

Biochemical testing

For identification of organism biochemical testing was done, using biochemical identification kit (HiMedia, Mumbai) for biochemical tests viz. catalase test, oxidase test, nitrate reduction, lysine utilization, glucuronidase, indole test, methy red test, Voges- Proskauer test, citrate utilization test, ortho- Nitrophenyl-β-galactoside (ONPG) test and sugar fermentation tests viz. lactose, glucose, sucrose, sorbitol. For biochemical testing isolated colonies on EMB agar and Mac-conkey agar were isolated and transferred aseptically to nutrient broth and after 7 to 8 hour of incubation the broth culture was subjected to biochemical testing and IMViC test (Indole, Methy red, Voges-Proskauer and citrate utilization test). According to cultural, morphological and biochemical characteristics bacterial isolates were identified (Cruickshank *et al.,* 1975).

Results and discussion

Gram staining procedure of overnight culture revealed, Gram Positive, Gram negative, rod, cocci and coccobacilli shaped organism. On Mac-conkey agar mucoid pink colonies and on EMB agar colony showing metallic sheen was observed. Colonies on mannitol salt agar and Edward medium were yellow and slightly bluish respectively. On biochemical testing of mac-cokey agar colonies IMViC pattern of of --++, and was positive for catalase, nitrate, ONPG, lysine utilization and negative for glucoronidase. On sugar fermentation test with following sugar *viz* lactose, glucose, sucrose and sorbitol only lactose and glucose showed positive result and was non motile. EMB agar colonies on biochemical testing revealed IMViC pattern of ++--, motile, and was positive for catalase, nitrate, ONPG, lysine utilization, glucoronidase. On sugar fermentation test with following sugar *viz* lactose, glucose, sucrose and sorbitol all showed positive result. Mannitol salt agar and Edward media colonies showed positive and negative catalase test respectively. Following Gram staining, colony characteristic and biochemical testing 24 isolates were identified in milk samples including major pathogen 10 (41.66%) *Staphylococcus,* followed by 7 (29.16%) *Streptococcus,* 4 (16.66%) *E. coli* and 3 (12.5%) *Klebsiella* organism.

The present finding are in accordance with the finding of Sumathi *et al.,* (2008) where they tested 60 milk samples out of which 40% *Staphylococcus,* 16 % *Streptococcus,* 20% *Escherichia coli,* and 10% *Klebsiella* organisms were isolated from milk samples. Mastitis caused by staphylococci is most prevalent and important in

context to India. *Staphylococcus* comes from unhygienic practices and via milkers hand. *E. coli* and *Klebsiella* origin takes place from contaminated environment and poor hygienic condition and it causes infection in udder via gaining entry through teat canal (Mallikarjunaswamy and Murthy, 1997). *Streptococcus* which multiplies in the milk, on the mammary epithelial surfaces, generally causing a subacute or chronic inflammatory reaction with periodic acute flare-ups and are obligatory parasite of mammary epithelial tissue. The affected tissue eventually is destroyed resulting in reduced milk production (Sharma *et al.,* 2012).

The mastitis milk tested in the study area is of poor bacteriological quality and hazardous for human consumption. For control of environmental mastitis hygienic practices of farm and environment should be adopted. These findings highlight the need to implement improved hygiene practices and to apply effective monitoring throughout the production to delivery chain. The pasteurization process and hygienic conditions at the milk production units along with cold chain of milk from suppliers to end users needs improvement.

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