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Case Report

Biofilm Associated Staphylococcal Mastitis in Goats

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Abstract

Staphylococcal mastitis is considered as an important cause of contagious mastitis. Staphylococcus aureus, a common such cause, has implications with regard to public health also. Biofilm formation with regard to Staphylococcus aureus plays an important role in antibacterial resistance. Treatment which is not effective in case of mastitis has severe economic repercussions for the country and for livestock farmers. The study reports on the occurrence of biofilm in Staphylococcus aureus mastitis in two Malabari goats. The goats were treated with Inj. Amoxicillin-Sulbactam @ 10 mg per kg intravenously along with Inj. Meloxicam @ 0.5 mg per kg intramuscularly for 7 days, reduction in inflammation was recorded, but clot formation was however present.

Keywords: Biofilm, Mastitis, Malabari Goat

Introduction

Mastitis has a huge economic impact with regard to the dairy industry and also for the individual livestock farmer. Mastitis in ruminants may manifest as acute, subclinical and chronic forms. The condition has varied etiologies and is often associated with bacterial infections. *Staphylococcus aureus* is an important etiological agent of mastitis, often causing contagious mastitis (Hamadani *et al.,* 2013). Nosocomial infections in humans, often attributed to medical implants, are often associated with *S. aureus* (Donlan and Costerton, 2002; Mathur *et al.,* 2006) underlines the relevance of research with regard to the bacteria and its biofilm. Treatment of infection with *S. aureus* is often challenging. Bacterial cure rate was reported as 52 per cent in clinical *S. aureus* mastitis in cattle (Sol *et al.,* 2000). Several factors play a role in the recurrence or re-infection and failure of treatment of mastitis in ruminants. It may include treatment for lesser number of days than warranted, treating without antibiogram and bacterial resistance. Bacterial biofilms are considered as an important factor associated with resistance to antibiotics. In the dairy industry, biofilms are recognized as a principal reservoir of microbial contamination (Parul *et al.,* 2019). Biofilm bacteria group together in a matrix of their own (Costerton *et al.,* 1999). Factors considered to play a key role in the mechanism of biofilm resistance are: protection by extracellular polymeric material, reduction in rate of growth and metabolism of bacteria and specific resistance which is conferred by the variation in the physiology of the biofilm bacteria (Davis, 2003). The present study reports on the occurrence of biofilm in *S. aureus* mastitis in two Malabari goats.

Materials and Methods

Two Malabari goats brought to the University Veterinary Hospital, Mannuthy, on two different occasions with the complaint of clots in milk were examined. Both goats were presented with moderate inflammation of the udder and large quantities of clot in the milk. The udder was painful to touch. Initial culture of the milk samples before treatment revealed the presence of *Staphylococcus aureus.* Based on culture and sensitivity, both the cases were treated with Amoxicillin-Sulbactam @ 10 mg per kg body weight intravenously along with Meloxicam @ 0.5 mg per kg body weight intramuscularly. A moderate reduction in inflammation was reported by the owner of goat one by day one of treatment and the owner of the second goat reported less pain on touching the udder by day three of treatment. Anti-inflammatory treatment was stopped on day two and day three of treatment in goat one and goat two respectively. By day five of treatment, a moderate reduction in the clots in milk was evident in both the cases. During the course of treatment, milk was collected again for biofilm assay. *Staphylococcus aureus* was present in culture. The treatment was continued for a total of seven days in the cases. Reduction in inflammation was recorded in both the cases. The clot formation had reduced considerably, but was however present. The owners of both the cases did not present the case after the said period.

For biofilm studies, the culture samples from the affected goats and *S. aureus*, SA113 (positive control) (Biswas *et al*., 2006) were grown in filter sterilized tryptic soy broth containing 1% glucose (TSB-G) at 37^0 C with 160 rpm shaking. For biofilm assay, Staphylococcal strains were grown in filter sterilized TSB-G in 96 well flat bottom plates. The biofilm assay was carried out (Biswas *et al*., 2006; Varma *et al.,* 2011). Biofilms formed in the wells were stained with 0.1% crystal violet. For quantification of biofilm formation, 100 µl of 70 per cent acetone was added to the wells to extract crystal violet and optical density was read at 580 nm using plate reader and plotted graphically. Biofilm formation of the samples was compared with standard strain of *S. aureus*, SA113.

Results and Discussion

All the samples formed biofilm in polypropylene surface. Biofilm formation was comparable between the standard strain of *S. aureus*, SA113 and that from goat 1. A much thicker biofilm was formed in the sample from goat 2 and retained more crystal violet. The optical density measurement indicated that the goat 2 sample produced about 1.4-fold thicker biofilm compared to standard strain of *S. aureus*, SA113.

The present study establishes the presence of biofilms in milk samples of goats from this geographical area. It is a major cause of concern as it has been found that bacterial biofilms resist the immune system of the patient and antibiotics. They are very resistant to sanitation procedures also (Song *et al.,* 2016). It has been reported that within host evolution of *S. aureus* results in enhanced biofilm production (Marbach *et al.,* 2019). *Staphylococcus aureus* has considerable public health concerns also as it is considered as a very important food borne pathogen (Hennekinne *et al.,* 2012). Further studies are warranted in this regard for a better understanding of the occurrence

of biofilms in goat mastitis, bacteria implicated, response to treatment and its public health concerns, especially with regard to the area from which the study was conducted.

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Conflict of Interests

There is no conflict of interest.

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