I. INTRODUCTION

Mastitis is an inflammation of the udder (mammary gland) and is common in dairy cattle. Mastitis is the most prevalent infectious disease of adult dairy cattle resulting in severe economic losses to the farming community. Several species of bacteria, fungi, mycoplasmas and algae have been incriminated as causative agents and also isolated from natural cases of mastitis. Bovine mastitis is one of the most problematic disease conditions and has got major economic impact on the dairy industry throughout the world. It is difficult to estimate the losses associated with clinical mastitis, which may arise from the cost of treatment, culling and decreased milk production (Bradley, 2002). However, the mastitis alone can cause approximately 70 per cent of all avoidable losses incurred during milk production. A world wide annual loss due to mastitis is around $ 35 billions and total losses accounts upto Rs.6053.21 crores per annum in India due to mastitis alone (Dua, 2001).

Variety of factors like mechanical trauma, thermal injury and chemical insults predispose the gland to intramammary infection. Occurrence of mastitis depends on the interplay of host, agent and environmental factors (Zhao and Lacasse, 2007). The classical mastitis pathogen is classified as contagious and/or environmental. The contagious pathogens are considered as organisms adapted to survive within the host, particularly in the mammary glands. They are capable of producing sub clinical infection which is typically manifested by elevation of somatic cell count (SCC) in the milk from the affected quarter. The organisms usually spread from cow to cow around or at the time of milking. In contrast, the environmental pathogens are best described as ubiquitous in nature and opportunistic
invaders of mammary gland. These organisms not adopted to survive in the host but produce clinical manifestations and are rapidly eliminated (Bradley, 2002).

The vast majority of infectious agents causing mastitis are of bacterial origin which include *Staphylococcus aureus* (*S.aureus*), *Streptococcus uberis* (*S.uberis*), *Streptococcus dysgalactiae* (*S.dysgalactiae*), *Streptococcus agalactiae* (*S.agalactiae*) and *Esherichia coli* (*E.coli*) accounts for almost 80 per cent of all subclinical mastitis cases (Anon., 2001; Ali *et al*., 2008).

In majority of bovine mastitis cases *Staphylococcus aureus* is a primary pathogen and is responsible for substantial economic losses in the dairy industry world-wide. The *Staphylococcus aureus* is one of the most frequently (45 % - 60 %) isolated pathogens (Verma, 1988; Kaya *et al*., 1998; Wani and Bhat, 2003 and Ali *et al*., 2008) and causes either clinical or subclinical or chronic bovine mastitis. In cows, intramammary infections (IMI) due to *S.aureus*, are generally subclinical which account for 25-30 per cent of cases and mastitis impairs alveolar function, reduces milk yield and affects chemical composition of milk, one of which is an increase in milk SCC (Leitner *et al*., 2000; Dego and Tareke, 2003 and Ali *et al*., 2008).

The treatment of mastitis necessitates extensive use of antibiotics in dairy herds. But increasing public concern over food safety, the governing authorities are making efforts to minimize antibiotic residues in milk. Moreover, the presence of *S.aureus* in raw milk used by dairy industries also poses threat to public health (Leitner *et al*., 2008).
The mastitis could be subclinical, which may be undetectable by the use of diagnostic tests applied to the milk or its secretion. The treatment cost of subclinical mastitis is very difficult to quantify, but most experts agree that the subclinical mastitis costs more than the clinical mastitis for an average farmer. About 70 percent of losses due to mastitis are associated with a reduction in milk production and a large portion of it results from irreversible damage to the mammary tissue (Oliver and Calvinho, 1995).

“Biofilms (BF) are microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a self produced matrix of extracellular polymeric substances / exopolysaccharide matrix and exhibiting an altered phenotype with respect to growth rate and gene transcription” (Costerton et al., 2003). Within a BF, bacteria are able to interact with each other through intercellular communication and thus rapidly adapt to changing environments. Biofilms adopt their own strategy of survival by way of altering their cell wall proteins and other components and escapes from host defense system. Microcolony or sessile bacterial cells under BF mode of growth may express low level of antigenicity, stimulating immune response and induce antibody production. However, these low levels of antibodies may not be effective in killing bacteria inside the BF. Therefore, by stimulating the immune response effectively by exogenous administration of BF antigens, early humoral responses can be induced against the exopolysaccharides responsible for biofilm formation, thereby avoiding the appearance of these micro colonies and controlling the infections associated with BF or preventing establishment of BF on the mucosal or epithelial surfaces. This could be achieved by in vitro growth of the bacteria in BF mode, which simulate natural in vivo
conditions to express novel immunogenic proteins. Hence, such BF grown bacteria can be exploited as potential vaccine candidate against mastitis causing organisms in bovines.

Intramammary infections (IMI) caused by \textit{S.aureus} in bovines are very difficult to cure. In the context of the high prevalence and economic consequences of \textit{S.aureus} IMI and the relative inefficiency of control measures, the development of a vaccine against \textit{S.aureus} IMI is of great interest. Vaccination has been employed as an adjunct to therapy as well as a preventive measure for \textit{S.aureus} mastitis. Several vaccines have been formulated based on bacterial cell wall components (protein A), adhesion factors (bacterial factors that allow \textit{S. aureus} to attach to mammary epithelial cells) and \textit{S.aureus} pseudo capsules which have been evaluated for protection against \textit{S. aureus} mastitis (Pamela and Ruegg, 2001). The outcomes of these studies have been inconsistent and confusing. Although, \textit{S. aureus} bacterins (Somatostaph®/Lysigin®) and ‘Mastivac I’ (Leitner \textit{et al.}, 2008) are commercially available in the United States of America (USA) and Israel respectively, have limited ability to prevent new IMI infections. Experimental vaccines for \textit{S. aureus} composed of pseudocapsule-enriched bacterins supplemented with \(\alpha\) – and /or \(\beta\) - toxoids appear promising, but none of these has been commercialized (Yancey, 1999). Many other conventional vaccines are also commercially available against \textit{S.aureus} mastitis. The efficacy of such vaccines in reducing the severity of clinical disease has been demonstrated (Nordhaug \textit{et al.}, 1994a; Giraudo \textit{et al.}, 1997; Leitner \textit{et al.}, 2003b and Lee \textit{et al.}, 2005) but the vaccines seem unable to prevent new intramammary infections. As yet, no commercial vaccines are currently available in India and other developing countries and it is unlikely that vaccines themselves will give the whole answer to bovine mastitis in future days.
Rabbits have been considered to be a good animal models for mastitis studies as the lactating mammary gland of the rabbit is susceptible to natural infection by Staphylococci and the disease could be reproduced in the laboratory by injecting low numbers of organisms into the mammary tissue. The type of disease produced varied with the strain used to infect the lactating mammary gland in rabbits (Adlam et al., 1977 and Adlam et al., 1980). Further, they are economical and have a greater number of teats than ruminants. Hence, they may help to reduce the cost and the number of animals involved. They are also larger than mice and may thus be easier to handle them for intramammary injections or infusions (Amorena et al., 1991 and Reinoso et al., 2002) and collection of milk.

Keeping this information in background, the present study was designed to study the pathology of experimentally induced Staphylococcus aureus mastitis and to evaluate the immune responses against Staphylococcus aureus biofilm vaccine against mastitis in rabbits. The study was designed to include the following objectives:

**Objectives of the study:**

- To induce experimental mastitis in rabbits by pathogenic *Staphylococcus aureus* isolated from bovine mastitis cases
- To study the gross, histopathology including immunohistochemistry in experimentally induced mastitis by pathogenic *Staphylococcus aureus* in rabbits
- To study the gross, histopathology including immunohistochemistry in rabbits immunized with biofilm and free cell vaccines and challenged with pathogenic *Staphylococcus aureus*