Mastitis is a most common and quite damaging disease in milking animals worldwide, with a significant economic influence on dairy industry. Concerted efforts for eradication or reduction of incidence of mastitis are going on for the last two decades, but still it remains a major threat to the dairy industry causing a huge economic loss. In 2002, losses due to mastitis have been estimated approximately US $35 billion globally (Wellenberg et al., 2002). Currently, the projected annual losses caused by mastitis to dairy farmers in US and UK are reported approximately US$2 billion and £300 million, respectively (Viguier et al., 2009). In Northern Ireland, the cost of clinical mastitis for an average 100 cow herds is £5000 per year and total mastitis infections costing of £14 million annually. In Republic of Ireland, the cost of clinical mastitis is approximately €693 per year for every infected cow. In the Netherlands, the average cost per infected cow varies €164 to €235. The average expenditure in treatment of a clinical mastitis case has been reported US $179 per lactating season (Bar et al., 2008). In India, 50% of milch animals are affected with mastitis, out of which clinical mastitis accounted for only 10%, and this costs an annual economic loss of ₹160720 million (Naresh and Varshney, 2005).

Additionally, mastitis has also been reported to be the third leading reason for premature culling of dairy animals followed by low yield and reproductive problems, as the primary reasons for disposal (Shook, 1989). The losses are due to veterinary measures, reduced milk production in the remaining lactation, milk discarded due to antibiotic treatment, early culling, extra labour, and decreased milk quality (Pösö and Mantysaari, 1996; Hogeveen and Østerås, 2005). Moreover, due to complexity of this disease losses are increasing and various reasons exist (incomplete perfusion of the antibiotics in bovine neutrophils, hidden capability of microbes in udder abscesses, antibiotic-resistance in isolates, etc.) for the failure of treatment and eradication of microbial mastitis from herds (Bayles et al., 1998). Complete eradication of mastitic pathogens from herds seems to be very difficult (Kerr and Wellnitz, 2003).

Different kinds of microorganisms e.g. *Staphylococcal* sp., *Streptococcal* sp., *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Corynebacterium bovis*,...
Corynebacterium pyogenes, Bacillus sp., Mycoplasma bovis and Micrococcus sp., etc. are common etiological agents of mastitis in animals (Forsman et al., 1997; Wilson et al., 1997; Sori et al., 2005; Lee et al., 2008). However, the major part of microbial mastitis has been reported due to staphylococci and streptococci (Phuektes et al., 2001; Gillespie and Oliver, 2005). Amongst these causative agents, Staphylococcus aureus has been recognized as the most common cause of intra-mammary infection in milch animal species, which often leads towards the damage and sometimes even complete loss of the gland. Staphylococcal mastitis has been reported as 25-30% of the entire mastitic infections and milk losses, which has been reported to vary between 10 to 25% (Sutra and Poutrel, 1994).

The initiation of intra-mammary infections by S. aureus is mediated through various virulence factors. Strains of S. aureus have been reported to produce numerous microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) or adhesins (protein A, clumping factor, fibronectin, fibrinogen, elastin, collagen, bone sialoprotein, laminin, thrombospondin, pseudo capsule ligand binding proteins, etc.), enzymes (hemolysins, coagulase, nucleases, and collagenase), enterotoxins and exfoliative toxins (Sutra and Poutrel, 1994; Foster and Höök, 1998; Zschöck et al., 2004; Reinoso et al., 2008; Campoccia et al., 2009; Ikawaty et al., 2010). MSCRAMMs are imperative for S. aureus which enable this pathogen in attachment, colonisation, invasion and infection in host (Patti et al., 1994; Foster and Höök, 1998).

Protein A is an important MSCRAMM of S. aureus produced during cell wall synthesis and plays a vital role in host defence evasion. This MSCRAMM has a unique property of attachment with the Fe fragment of immunoglobulin G (IgG) in various species including bovines (Goudswaard et al., 1978). It’s anti oposonic activity gives the power to resist phagocytosis and increases virulence of S. aureus and acts as an immunological disguise. Protein A has been used as a potential marker to discriminate epidemic and non-epidemic isolates of S. aureus (Montesinos et al., 2002; Reinoso et al., 2008). Clumping factors A and B also remain other important cell wall components of S. aureus as these help in binding with fibrinogen (Foster, 2005). Fibronectin-binding protein A and B mediate adherence to fibronectin, while coagulase induces polymerization of fibrinogen to fibrin. Additionally, fibrinogen-binding protein promotes attachment to fibrinogen and elastin-binding protein mediates binding to
soluble tropoelastin (Bodén and Flock, 1989; Downer et al., 2002). Collagen binding proteins contribute to form a connection with collagen. MHC class II analog protein (Map) is a crucial cellular protein having broad binding activity to fibrinogen, collagen, fibronectin and elastin, which enable colonization in different tissues. Adherence of *S. aureus* to fibroblasts and epithelial cells increase significantly with the expression of Map (Peacock et al., 2002). Capsular polysaccharide (Cap) of *S. aureus* helps to resist phagocytosis and Cap type 5 and 8 have been reported predominantly in mastitic isolates (Akineden et al., 2001; El-Sayed et al., 2006; Reinoso et al., 2008).

Similarly, the staphylococcal toxins (enterotoxins, exfoliative and toxic shock syndrome) have been reported to modulate immune response through the super-antigenic activity leading to various diseases (Peacock et al., 2002). Alpha-hemolysin is dermonecrotic, neurotoxic, and lyses mammalian cells, especially red blood cells, by forming pores in the target membrane (Bhakdi and Tranum-Jensen, 1991). Beta-hemolysin acts as sphingomyelinase and gamma-hemolysin shows leucocytolytic activity. In addition, delta-hemolysin has surfactant or channel forming properties (Dinges et al., 2000). *S. aureus* strains produce additional exoproteins, which might have host defence evasion as their major functions reported *in vivo* models. Moreover, the information is still scanty on the role of staphylococcal MSCRAMMs and toxins in mastitis pathogenicity (Annemüller et al., 1999; El-Sayed et al., 2006). Moreover, the expression of virulence factors varies among strains of *S. aureus* that leads to variability in pathogenicity of this microorganism (Haveri et al., 2007; Fournier et al., 2008). There always remains a need for effective control of bovine mastitis and strain typing to identify the degree of virulence and the distribution of virulence factors like MSCRAMMs in the isolates of *S. aureus* from subclinical and clinical stages (Patti et al., 1994).

The antibiotic-resistance of *S. aureus* strains is another serious concern besides the pathogenicity. The emergence of antibiotic-resistance in *S. aureus* mastitic dairy animals has been shown in recent years (Moon et al., 2007; Hendriksen et al., 2008; Wang et al., 2008). Strains of *S. aureus* are reported to show resistance against multiple antimicrobials (Wang et al., 2008). Various genetic determinants such as MecA (methicillin), *TetK/M* (tetracyclines), *MsrA/B* (macrolides), *AacA-D* (aminoglycosides), *ErmA/B/C* (macrolide-lincosamide-streptogramin B) and *LinA*
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Lincosamides resistance) have been reported for resistance mechanisms in S. aureus isolates (Lina et al., 1999b; Wang et al., 2008; Turutoglu et al., 2009). Among the various antibiotic-resistant isolates, methicillin-resistant S. aureus (MRSA) is a serious cause of concern in both human and animals (Türkyılmaz et al., 2010). In many cases, colonization or infection by MRSA in animal species appears to be due to zoonotic transfer from human owners or caretakers (Smith and Pearson, 2010). MRSA infections are not easy to be treated. These genomic components enable S. aureus to reside for a long time inside the host and herd environment, and often create hurdles in treatment.

Genetic variants of S. aureus and their relationship with the geographical regions have been studied and the genotypic analysis emphasis that only a few strains of S. aureus can cause bovine mastitis in each region (Fitzgerald et al., 1997; Annemüller et al., 1999; Zschöck et al., 2004). Diversity in the expression of virulence factors could cause changes in the level of pathogenicity, sustainability and spreading of the infections within, and between animals (Haveri et al., 2007; Herron-Olson et al., 2007; Fournier et al., 2008; Capurro et al., 2010). Hence, the evaluation of the polymorphic nature of the genetic determinants of such virulence and antibiotic-resistant traits can further contribute to the construction of a bacterial population genetic framework. This information can be relevant to the pathobiology of S. aureus mastitis in different regions. The identification of virulence factors and antibiotic-resistance of the mastitis causing isolates is possible using the molecular tools.

The changes in epidemiology of mastitis in recent years have put emphasis to elucidate udder immune system in the pathogenesis of S. aureus. The genes controlling immune response to specific antigens, like that encoding for the immunoglobulin allotype, have been reported probably associated to a number of diseases (Dugoujon and Cambon-Thomsen, 1995; Kameda et al., 1998). Gene organization of the immunoglobulin loci has been extensively analyzed in human populations (Grubb et al., 1990; Balbin et al., 1994). Among the four immunoglobulin systems (Gm, Am, Em and Km), the Gm system has shown the greatest degree of polymorphism and is thus most often studied in population genetics of human as well as animals (van Loghem et al., 1982; Rabbani et al., 1997). As the high degree of polymorphism in this region exists in populations, it is the speculation that particular Gm allotypes might be associated with genes that confer selective advantages. Conversely, particular Gm
allotypes might be predisposing an individual to disease (Whittingham and Propert, 1986). Previous studies have shown associations between Ig allotypic phenotypes and susceptibility to several diseases in human populations (Herrera et al., 1996). In bovine, Rabbani et al. (1997) cloned and analyzed the highly polymorphic IgG3 gene, which differs most remarkably from IgG1 and IgG2 in structure of its hinge and revealed different alleles. This leads to the possibilities of applying the polymorphic genetic systems such as immunoglobulin structural gene and their allotypes as candidate markers for genetic characterization of animals reflecting their genetic predisposition to diseases like mastitis. It has been shown by several studies that genetic variations exist for resistance or susceptibility to diseases (Lin et al., 1989; Uribe et al., 1995). A proportion of this genetic variation can be explained by polymorphism at each locus affecting traits (Sharif et al., 1998).

Polymorphism of genes related with mastitis and their association with this disease have been reported in cattle. However, investigations on these genes in indigenous cattle and buffalo breeds are scanty. Besides, there is lack of information on association between mastitis and polymorphism of candidate genetic markers for resistance corresponding to bacterial infection. The investigations on the role of potential mastitis resistant/susceptible genes especially Ig allotypes in indigenous cattle and buffaloes might be an effective means of marker-assisted selection against staphylococcal mastitis. In view of the aforementioned knowledge and paucity of documented information on these aspects for indigenous Sahiwal and Karan Fries cattle, and the most important Murrah breed of buffalo, the present work has been carried out under the following objectives:

- To evaluate the distribution of genetic determinants of antibiotic-resistance in *Staphylococcus aureus* originated from animal mastitis
- To assess the role of *Staphylococcus aureus* MSCRAMMs in animal mastitis.
- Determination of the prevalence of toxin genes in *Staphylococcus aureus* originated from animal mastitis
- To delineate the staphylococcal protein A gene polymorphism in mastitic cattle and buffaloes.
- Evaluation of the Immunoglobulin G3 as a candidate genetic marker corresponding to staphylococcal animal mastitis.