Milch animals with high milk yield and improved health are key targets of dairy sector over the past years. Yet, the diseases like mastitis and reproductive failures in animals are causing huge economic losses in dairy industry. Mastitis, the inflammation associated with apoptosis of mammary epithelial cells has a considerable impact in averting the full genetic potential of an animal to produce milk. Multiple factors are responsible for mastitic infection in milking animals.

*S. aureus* has been recognized as the most common cause of IMI and microbial mastitis in milking animals. Pathogenesis in IMI includes various stages such as entry, attachment, evasion of defense system, survival and tissue invasion of the mammary glands, which is mediated by MSCRAMMs, cellular enzyme, exfoliative toxins and enterotoxins. Pathogenic factors also help to resist the opsonisation, and immune actions of host and antibiotics therapy. Antibiotic-resistance in *S. aureus* is also serious concern besides the pathogenicity, as infections by such strains are not easy to be treated and reside for a long time inside the host and herd environment. Antibiotic-resistance of *S. aureus* is emerging due to clonal evolution, mutation, and horizontal gene or plasmid transfer. Genetic determinants such as *MecA*, *TetK/M*, *MsrA/B*, *AAC-4* and *ErmA/B/C* and *LinA* are crucial for antibiotic-resistance mechanisms in *S. aureus* isolates. Moreover, diversity in the expression of pathogenic factors and antibiotic-resistance could change in the level of pathogenicity, sustainability and spreading of the infections within, and between animals. Therefore, the evaluation of virulence and antibiotic-resistant genes can be used to construct a bacterial genetic framework, and identify the degree of virulence and the distribution of pathogenic factors in *S. aureus* from different infection stages.

The immune system of udder and genes (immunoglobulin allotypes, TLRs, etc.) have been found associated to a number of microbial diseases. Immunoglobulin system shows the greatest degree of polymorphism, which has the possibilities to be used as candidate markers for genetic characterization of animals reflecting their predisposition to mastitis. Hence, the present work was designed to evaluate the role of putative pathogenic factors, and antibiotic-resistance of *S. aureus* and potential role of IgG3 allotypes in mastitic animals.
Screening of milking animals (555) of cattle (Sahiwal and Karan Fries) and buffaloes (Murrah) from NDRI, Karnal was carried out using the CMT, pH and chloride content tests for mastitis infection. The variable level of infection was observed among the animals and high infection rate was found in Karan Fries as compared to Sahiwal and Murrah. The infection of animals was categorized as clinical and subclinical on the basis of screening test. Based on CMT score, the most infected teats were LF (55) followed by RF (52) in Sahiwal cattle; whereas RH (56) and LF (50) remained in Karan Fries. Incidences of strong infection in Murrah were found in RF (21) and RH (19). The chloride content was low and high corresponding to the CMT scores. The pH range was high as compared to normal (7.5-7.7) in the strongly positive CMT cases and 6.6-6.8 in CMT negative. The milk samples were collected from most infected teat.

The infected milk samples showed presumptive colonies of staphylococci on Baird Parker agar. Among the 555 samples tested, 346 presumptive strains of *S. aureus* (Sahiwal 107, Karan Fries 128 and Murrah 111) were obtained. The recovered presumptive isolates of *S. aureus* were characterized for carbohydrate fermentation, which revealed positive assays for dextrose, galactose, glycerol, ribose, maltose, mannose, sucrose, fructose, trehalose, malonate, lactose, citrate, esculin and o-nitrophenyl-β-D-galactopyranoside. Additionally, isolates showed atypical patterns for mannitol (79.4%), rhamnose (72.6%) and glucosamine (55.6%) fermentation. The arginine and Vogues-Proskauer tests were found to be 75.9% and 66.7% in isolates. Further, identification was confirmed with 16S rDNA gene amplification and showed that all the obtained isolates (346) belonged to *S. aureus*. All the tested isolates screened for pathogenic factors were found positive for bio-film and capsule formation. Urease (97.2%), lysostaphin activity (95.2%), clumping factor (92.3%), deoxyribonuclease (86.9%), coagulase (85.8%), slime production (76.8%) and β-hemolysin (76.7%) activities were also observed in large proportion of isolates. However, occurrence of α-hemolysin (23.2%) and thermonuclease (60.8%) remained in low proportion as compared to other virulent factors.

The isolates tested for antibiotic-resistance revealed different patterns. A significant proportion of isolates (27.2%) was susceptible to all the antibiotics used. The susceptibility proportions were lower in Karan Fries isolates (15.6%) as compared to Sahiwal and Murrah.
Summary

to Sahiwal (31.8%) and Murrah (36%). Additionally, all the isolates remained susceptible to vancomycin and a huge proportion was also susceptible to cephalexin, ofloxacin, clindamycin and lincomycin. The resistance to antibiotics was higher in isolates of Sahiwal and lower in Murrah. Higher prevalence of MRSA (9.5%) was observed in all the tested isolates. The overall resistance in all the isolates remained high to tetracycline (33.8%), gentamicin (28%), kanamycin (27.5%), streptomycin (27.5%), erythromycin (24.6%) amikacin (24%), penicillin-G (22.3%), ampicillin (19.7%), ciprofloxacin (18.2%), cloxacillin (17.6%), and amoxicillin (17.1%). Antibiotic-resistance was higher in the strains obtained from clinical as compared to subclinical cases. The proportion of resistance to antibiotics, namely amikacin (38.5%), kanamycin (32.7), pencillin-G (32.4%), streptomycin (31.3%), erythromycin (29.3%) and ampicillin (25%) considerably dominated in clinical isolates. Similarly, prevalence of MRSA was higher in clinical isolates (16.1%) from Sahiwal followed by Karan Fries (15.5%) and Murrah (9.1%).

Molecular assessment of antibiotic-resistant determinants in isolates revealed predominance of \textit{Msr}A, \textit{Msr}B, \textit{Lin}A, \textit{Tet}K and \textit{Aac}A-D genes and 69.3% of tested strains expressed at least one kind of gene. All the isolates did not show \textit{Vat}B gene on amplification. \textit{Lin}A (54.3%) was the most frequent as compared to other genes. The distribution of \textit{Msr}B (46.5%) was more as compared to \textit{Msr}A (30.3%). Only, 7.5% of isolates revealed the \textit{Mec}A gene fragment. Moreover, 27.2% of isolates showed \textit{Aac}A-D gene. Among the tested isolates occurrence of \textit{Tet}K (32.1%) was significantly higher than \textit{Tet}M (4.1%) gene. The unusual appearance and low proportion of some genetic determinants (\textit{Vat}A, \textit{Vat}C, \textit{Erm}A and \textit{Erm}C) was also observed among the tested isolates. Distribution of antibiotic-resistant genes remained low in Murrah isolates as compared to Sahiwal and Karan Fries. The overall comparison revealed higher prevalence of genetic determinants in clinical than the subclinical isolates. The genetic determinants, namely \textit{Lin}A (74.3), \textit{Msr}B (65.7%), \textit{Tet}K (42.1) and \textit{Aac}A-D (37.9%) were more common in clinical as compared to subclinical isolates. The proportion of \textit{Mec}A gene was considerably higher in clinical (12.1%) rather than subclinical isolates (4.4%). Conversely, occurrence of \textit{Msr}A gene was slightly higher in subclinical (46.1%) than clinical strains (42.1%). Statistical correlation was observed to be significant ($P<0.05$) between \textit{Mec}A and \textit{Aac}A-D, \textit{Tet}K, \textit{Msr}A, \textit{Msr}B, \textit{Lin}A genes.
Association of AacA-D with MsrA, MsrB and LinA was also significant. Similarly, association of TetK, MsrA, MsrB and LinA was significant.

Evaluation of *S. aureus* strains for different pathogenic attributes showed high proportions of genes encoding Coa (94%), ClfA (94.5%), Ig-binding (100%), and Agr (100%). The proportions of different amplicons for the Ig-binding gene were 500 (14.7%), 1000 (33.5%) and 1050bp (51.5%), and for the ClfA were 950 (22.5%) and 1000 (41.6%), 1050 (20.8%) and 1100bp (6.4%). PCR-RFLP of Coa gene generated six patterns, namely A, B, C, D, E and F which were distributed in isolates at 37.3, 12.4, 13.3, 19.7, 5.8 and 5.3%, respectively. The genotypes B, C and F (31.2%) were more prevalent in subclinical *S. aureus* isolates. Whereas, A and D (57%) were more common in clinical strains. The occurrence of Agr-I and II (72.3%) was significantly higher than the Agr-III and IV (27.7%).

All the tested isolates were positive for virulence genes FnbA, Eno, Hla, Nuc, and prevalence of Map (81.7%), Fib (61.8%), Cap5 (59.5%) and Ebp (57.5%) was also higher in tested isolates. Genetic determinants FnbB (19.4%), Bhp (5.5%) and Cap8 (22.5%) were observed in lower proportion in isolates. Cap5 and 8 were not found in 18% isolates. None of the isolates showed superantigen Sea, Seh, Sej, Tsst-1, Eta and Etb on amplification. Enterotoxin gene Sei (47.1%) was the most frequent followed by Seg (12.7%) and Sec (7.2%) in tested isolates; whereas prevalence of Seb, Sed, and See remained very low in isolates. Statistical correlation was significant to be \((P<0.05)\) between Hlb and Fib, Ebp, FnbB and Map. Similarly, the presence of Bhp gene was associated with the isolates revealing Ebp. Correlation between FnbB and Cna was highly significant \((P<0.01)\). The presence of Sec in isolates was found significantly associated with Seg and Sei. Correlation between Seg and Sei was also found to be significant. The distribution association revealed that most of genes of pathogenic factors were frequent in Agr-I and II revealing isolates. The isolates expressing Ebp (72.8%), Fib (73.8%), Cap5 (72.9%), Map (71.4%) and Hlb (70.7%) genes were prevalent with Agr-I and II. The enterotoxins (Sec, See, Seg and Sei) genes were also considerably associated with Agr-I and II. However, Cap8 (68%), FnbB (70.2%), and Cna (82.4%) genes were associated with Agr-I and III subtypes. The overall distribution of pathogenic factors and superantigen determinants was found to be significantly higher in clinical isolates as compared to subclinical. The prevalence of
Summary

Hlb (100%), Map (90.7%), Fib (73.6%), Cap5 (65%), Ehp (61.4%) FnHB (28.6%) and Cap8 (26.4%) genes was higher in all the clinical isolates. Similarly, the occurrence of toxins genes Seb (2.1%), Sec (10%), Sed (4.2%) and Sei (60.7%) were significantly prevalent in clinical isolates as compared to subclinical.

Staphylococcal protein A showed polymorphism (3-12R) in the isolates. The highest frequency of 7R (39.3%) and 8R (39.3%) was found in isolates. The proportions of 4R (0.3%), 12R (0.6%) and 3R (0.9%) were found lower in all the tested isolates. The occurrences of 6R, 10R and 11R were also observed lower as compared to 9R. The overall occurrence of SpA>7R (55.5%) was more as compared to SpA≤7R (45.5%). The overall percentage in clinical isolates expressing SpA>7R (58.6%) was more as compared to SpA≤7R (41.4%). In subclinical isolates, 7R (41.7%) were the most frequent repeats. Whereas, in clinical isolates, 8R (40.7%), 9R (10.7%) and 10R (3.6%) were observed more common. Most of the isolates expressing SpA≥7R were observed to show larger fragments of ClfA and Ig-binding genes. A significant proportion of isolates showing 7R (84.6%) was found to reveal the larger amplicons (1000 and 1050bp) of Ig-binding genes. The isolates showing 3R and 4R harbored the smaller fragments of Ig-binding and ClfA. The proportion of coagulase genotypes A and D were observed higher in isolates expressing SpA ≥7R and B, D, E and F were not found in isolates with 3R, 4R and 12R in SpA. Similarly, Agr subtypes I and II predominated in isolates showing SpA ≥7R. The Agr-III remained higher (66.7%) in isolates showing 3R. All the isolates with SpA ≥7R were found to express large number of pathogenic factors when compared with other categories. Isolates expressing 7R, 8R, 9R, 10R showed more number of genes.

The distribution of IgG3 allotypes studied in 152 cases (Sahiwal, Karan Fries and Murrah) that were selected on the basis of their mastitis history and categorized in two groups. The first group included the random mastitic cases (116) and second group of 36 were positive for IMI caused by *S. aureus*. The detection of the allotype in IgG3 was carried out using PCR-RFLP. Among the 30 screened endonuclease enzymes, amplicons revealed polymorphism with BsaAI, BstYI and HphI. The alleles D (0.347), A (0.400) and E (0.400), and genotypes BD (0.500), BD (0.375), DE (0.600) generated by BsaAI were found in higher frequency in Karan Fries, Sahiwal and Murrah animals, respectively. In second group, alleles B (0.357), A (0.553) and E (0.350) were present.
in greater frequency in animals of Karan Fries, Sahiwal and Murrah, respectively. Genotypes BD (0.428), AA (0.421) and DE (0.600) were found to be highest frequency in the order. Allele A generated by BstYI was found to be 0.500, 0.550, and 0.700; whereas genotype AC (0.722), AB (0.675) and AA (0.400) was the highest genotypic frequency in Karan Fries, Sahiwal and Murrah, respectively. Similar observation was resulted in second group. Allele A revealed by Hph1 digestion was present in higher frequency in all the breeds and AC and AB were the highest genotype frequency. In second group, allele A was also found to have higher frequency 0.643, 0.500 and 0.500 in Karan Fries, Sahiwal and Murrah, respectively. Heterozygous AC was found to be highest (0.428 and 0.800) genotype frequency in Karan Fries and Murrah, respectively. But, genotype AB was observed to be higher (0.684) frequency in Sahiwal animals. Statistical value of Chi-square was significant ($P \leq 0.001$ at 3 degrees of freedom) in tested animals and showed that population was not in Hardy-Weinberg equilibrium.

Genotypic analysis revealed that genotypes AB, AC and DE (BsaAl) remained higher in affected cases of Karan Fries and Sahiwal and BD in unaffected ones. In Murrah animals, EF remained higher (07 cases) in unaffected category and AA, BD and DE predominated in affected. Genotypes produced with BstYI did not vary significantly in unaffected and affected in Karan Fries. In Sahiwal and Murrah, unaffected category showed only AB genotype; whereas AC and AA were observed predominately in affected cases. Similarly, AB (Hph1) was common in unaffected cases and AC in affected animals of Karan Fries and Sahiwal cattle. In case of Murrah, AC was common in affected and unaffected animal and AB in affected cases only.

The genotypes of animals in second group correlated with SpA repeats showed AA (25%) in Karan Fries generated by BsaAl was associated with SpA≤7R and DE (33.3%) with SpA>7R. Similar patterns were observed in Murrah buffaloes. In case of Sahiwal, SpA≤7R was prevalently related with BD and DE (66.6%); whereas SpA>7R in AA (47.1%). The genotype AB (50%) produced by BstYI in Karan Fries was found to be correlated with SpA≤7R; whereas AC (66.7%) with SpA>7R. In Sahiwal cattle, AA and AB were more favorably associated with SpA≤7R and AB significantly (64.7%) associated with the SpA>7R. The genotype AB seemed influenced (100%) with SpA>7R and AC in SpA≤7R in cases of Murrah. Similarly, genotype AA (50%) generated by Hph in Karan Fries were more likely to be correlated with SpA≤7R, while

Summary
with \textit{SpA}>7R. In Sahiwal and Murrah, genotype \textit{AA} was absent. Genotype \textit{AB} and \textit{AC} were not apparently associated with \textit{SpA} as the animals consisting of these genotypes were infected with both kinds of strains.

Multiple sequence alignments were examined to identify variation in genotypes. The nucleotide sequences of genotypes \textit{AA}, \textit{AB} and \textit{AC} (generated by \textit{HphI}) showed changes at position 245 and 256 (A to G) in genotype \textit{AB}, and A to T at 327 and 338 was changed in \textit{AC} genotype. The changes at 245, 256 and 327 resulted from valine to glutamate, aspartate to arginine and arginine to proline substitution. Genotypes \textit{AB}, \textit{BD} and \textit{DE} (\textit{BsaAI}) revealed amino acid changes at 294 (valine to leucine), 719 (aspartate to glycine) and 809 (cysteine to tryptophan). Similarly in genotypes by \textit{BstYI} showed changes at 35 (leucine to phenylalanine), 174 (isoleucine to proline), 291 (aspartate to isoleucine), 372 (isoleucine to glycine), 456 (serine to proline). Some nucleotide changes in the deduced amino acids sequence were not detected.

Eventually, genetic components, namely \textit{Hla}, \textit{Eno}, \textit{FnB}, \textit{Ebp}, \textit{Fib}, \textit{Map} and \textit{Nuc} unequivocally appeared to be responsible in establishment of mastitic infection (subclinical and clinical) as expressed by almost all the isolates. The distribution of high pathogenic characteristics in antibiotic-susceptible isolates indicated these were equally responsible in maintaining IMI in animals and cannot be overlooked. The assessment of pathogenic and antibiotics resistance factors of isolates revealed that certain genetic elements were over producing (\textit{Ebp}, \textit{Fib}, \textit{FnB}, \textit{Bbp}, \textit{Map}, \textit{TetK}, \textit{MsrB}, \textit{AacA-D}) in mastitic \textit{S. aureus} isolates especially from clinical cases. This outcome or genetic frame of \textit{S. aureus} isolates may be further useful for culling or segregation of animals infected with harmful strains to reduce the dissemination of pathogenic microorganisms or containment of mastitis. Further, the present study identified the genotypes of IgG3 gene in mastitis resistance/susceptible animals and association with determinants of \textit{SpA} gene of \textit{S. aureus} strains. The present investigation will provide the vital information which may be useful in animal breeding evaluation strategies.