JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1055-1061

Characterization of *Staphylococcus aureus* of Goat Mastitis Milk Origin for *cap* and *clf*A Genes

Prerna Nathawat[1], Taruna Bhati[2], Sandeep Kumar Sharma[3]*, Rahul Yadav[2] and Anil Kumar Kataria[2]

[1]Centre for Studies on Wildlife Management and Health, College of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animals Sciences, Bikaner (Rajasthan), India.
[2]Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Bikaner Rajasthan University of Veterinary and Animals Sciences, Bikaner (Rajasthan), India.
[3]Department of Veterinary Microbiology and Biotechnology, Post Graduate Institute of Veterinary Education and Research (PGIVER)- Jaipur, Rajasthan University of Veterinary and Animals Sciences, Bikaner (Rajasthan), India.

(Received: 02 February 2015; accepted: 06 March 2015)

Staphylococcus aureus **is major pathogen causing mastitis in dairy milch animals including goats. The present study was carried out on 27 *S. aureus* isolates of milk origin from goats with clinical mastitis which included characterization of isolates for two important virulence associated genes namely *clfA*, responsible for clumping factor and *cap*, responsible for capsule. All the isolates were genotypically identified by 23S rRNA ribotyping in which a species-specific amplicon of 1250 bp was obtained. Of the 27 isolates 17 (68.38%) showed single amplicon of approximately 350 bp representing presence of *cap*5Kgene responsible for capsular type 5 whereas nine (34.61%) of the isolates produced amplicons of approximately 200bp representing presence of *cap*8K genes responsible for production of capsular type 8, whereas one isolate was non-typable for any of the two genes.In the present investigation each of the 25 isolates produced single amplicon of 1050 bp indicating no polymorphism in *clf*A genewhile two isolates did not produce any PCR amplicon. Epidemiological investigation of these genes is significant in terms of vaccine design and control of mastitis from the study area.**

Key words: *Staphylococcus aureus, cap, clf*A, goat, mastitis.

Goat is one of the oldest domesticated species which is used for milk, meat, hair, hide, and manure. It is very important animal species especially in western Rajasthan where most of the land area is arid and rearing is done almost on zero input. Mastitis is one of the important diseases of goat wherein due to improper or delayed diagnosis and no/or improper treatment, they are ultimately rendered useless for purpose of milk production. The disease is multi-etiological complex in which *Staphylococcusaureus* has been found to be the most important pathogen worldwide for clinical as well as subclinical forms in dairy small ruminants (Bergonier *et al.*, 2003; Aras *et al.*, 2012).

A variety of virulence factors are produced by this organism which are involved in the pathogenesis of mastitis (Poutrel *et al.*, 1988; O'Riordan and Lee, 2004; Momtaz *et al.*, 2010), however, all factors are not produced by each strain (Balaban and Rasooly, 2000). Capsular polysaccharides produced by *S. aureus*isolates are considered important virulence factors in the

* To whom all correspondence should be addressed.
Mob.: +91-9414775879;
E-mail: drsharmask01@hotmail.com

pathogenesis of Staphylococcal infections. Of the many capsular types cap5 and cap8 have been considered important in pathogenesis and hence in vaccine development (Risley *et al.,* 2007; Tuchscherr *et al.,* 2008). These capsular polysaccharides are surface bound and promote bacterial colonization and persistence on mucosal surfaces. Clumping factor A (ClfA) is a cell wall-anchored *S. aureus* surface protein that has been shown to enhance staphylococcal virulence in animal infection models (Moreillon *et al.,* 1995; Wolz *et al.,* 2002) and has been suggested as a vaccine potential candidate (Josefsson*et al.,*2001;Nour El-Din *et al.,* 2006) and as a target for passive immunization approaches (Domanski *et al.,* 2005; Patti, 2004; Vernachio *et al.,* 2003).

Both of the virulence factors have also been suggested to be potential vaccine candidates (Risley *et al.,* 2007). In one study, Tuchscherr *et al*. (2008) showed that antibodies to *clf*A enhanced the protection against infection provided by capsular polysaccharides antibodies. Caprine mastitis has been recorded in different countries by different authors (Moroni *et al.,* 2005; Ayedin *et al.,* 2009) but data from India are scarce. The present paper reports characterization of *S. aureus* of goat milk origin for capsular and clumping factor genes.

MATERIALS AND METHODS

Isolation and identification of bacteria

Isolation of *S. aureus* was carried out from milk samples of Marwari goats native to Bikaner with clinical mastitis. The organisms were isolated and identified as per the methods described by Cowan and Steel, (1975). Further they were genotypically identified by ribotyping based on 23S rRNA gene (Straub *et al.,* 1999) using species-specific primer-1 (5'-ACGGAGTTAC AAAGGACGAC-3') and primer-2 (5'-AGCTC AGCCTTAACGAGTAC-3'). The amplification products were electrophoresed for 1.5 h at 100 V and then gel was visualized using a UV transilluminator.

cap gene amplification:

The method of Verdier *et al*. (2007) was used for the amplification of *cap*5Kand *cap*8K genes using the primer sets: The sequences for two primers used for *cap*5K gene were: Primer-1: 5'GTCAAAGATTATGTGATGCTACTGAG3' and Primer-2: 5'ACTTCGAATATAAACTTGAAT CAATGTTATACAG 3', the sequences for two primers used for *cap*8K gene were: Primer 1: 5' GCCTTATGTTAGGTGATAAACC 3' Primer 2: 5' GGAAAAACACTATCATAGCAGG 3'. The PCR mixture (total volume 30 µl) was prepared by mixing 1.0 µl of primer-1 (75 pmol/µl), 1.0 µl of primer-2 (75 pmol/µl), 3.5 µl of 10x *Taq* buffer A (containing 15mM $MgCl_2$), 1 unit of *Taq* polymerase (5 U/µl), 2.0 µl of dNTP mix (10 mM/µl), 21.0 µl of deionised water, and 2.5 µl of DNA template (25 ng/µl). The PCR was performed using following cycling parameters: Cycle 1: Denaturation at 94°C for 5 min, Primer annealing at 55°C for 30 s, Primer extension at 72°C for 1 min; Cycle 2-25: Denaturation at 94°C for 30 s, Primer annealing at 55°C for 30 s, Primer extension at 72°C for 1 min; Cycle 26: Denaturation at 94°C for 30 s, Primer annealing at 55°C for 30 s, Primer extension at 72°C for 5 min.

clf A gene amplification

The method of Stephan *et al*. (2001) was used for the amplification of *clfA* gene using the primers set (Primer 1: 5'-GGCTTCAGTG CTTGTAGG-3'; Primer 2: 5'-TTTTCAGGG TCAATATAAGC-3'). The PCR mixture (total volume 30 µl) was prepared by mixing 1.25 µl of primer-1 (75 pmol/µl), 1.25 µl of primer-2 (75 pmol/µl), 2.5µl of 10x *Taq* buffercontaining $MgCl_2$ (25mM), 0.3 µlof *Taq* polymerase (5 U/µl), 2.5 µl of dNTP mix (10 mM/µl), 17.20 µl of deionised water and 2.5 µl of DNA template (25 ng/µl). The PCR was performed in Palmcycler (Corbett Research, Australia) using following cycling parameters:- Cycle 1: Preheating at 94° C for 4 min; Cycle 2-36: Denaturation at 94°C for 1 min, Primer annealing at 57°C for 1 min, Primer extension at 72°C for 1 min; final extension at 72°C for 5 min.

The PCR products, after addition of 2 µl of trekking dye were resolved in 1.2% agarose gels prepared in 1.0 x TBE buffer containing 0.5µg/ml of ethidium bromide and 100 bp DNA ladder was used as molecular marker. The amplification products were electrophoresed for 1 h 30 min at 100 V. The gel was then visualized under U.V. transilluminator.

RESULTS AND DISCUSSION

23S rRNA gene based confirmation

All the 27 isolates on 23S rRNA ribotyping produced a single PCR product of 1250 base pairs confirming them to be *S. aureus.* This method was found 100% specific in genotypic identification of *S. aureus*.

cap genotyping

Seventeen of the 27 (68.38%) isolates showed single amplicon of approximately 350 bp representing presence of *cap*5Kgene responsible for capsular type 5 (fig. 1) whereas nine (34.61%) of the isolates produced amplicons of approximately 200bp representing presence of *cap*8K genes responsible for production of capsular type 8 (fig. 2). In our study one of the 27 isolates was non-typable for CP5 or CP8.

clf A genotyping

In the present investigation 25 isolates produced single amplicon in each isolate whereas two of the isolates did not produce any PCR amplicon. The size of the amplicon product obtained in the present study was 1050 bp indicating no polymorphism in *clf*Agene (fig. 3).

Mastitis is one of the most important diseases of dairy animals globally leading to severe financial losses to dairy industry. Among dairy animal species goat has its own important place in many of the countries. In India also goat rearing is practiced by marginal farmers for their livelihood. *Staphylococcusaureus*has been identified as the most important udder pathogen in small ruminants (Bergonier *et al.,* 2003). This organism doesn't demonstrate host specificity and is responsible for many different infections in animals as well as humans. Aires-de-Sousa *et al.*(2007) characterized *S. aureus*from buffalo, cattle, sheep and goat milk samples by phenotypic and genotypic methods and they concluded that a major clonal type is responsible for mastitis in different regions which does not demonstrate host specificity among the different animal species and seems to be capable of both infection and colonization. Mastitis caused by *S. aureus* should be eliminated because of the risk of contamination of milk products by the thermo-stable toxins (Contreras *et al.,* 2007).

The identification of *S. aureus*strains is important to confirm the epidemiological relationships among them (Aarestrup *et al.,* 1994). All the isolates in the present study were identified by genotypic method in which 23S rRNA gene was targeted using specific primers. This method is being used for confirmation of *S. aureus* from different sources worldwide (Akinede*et al.,* 2001;

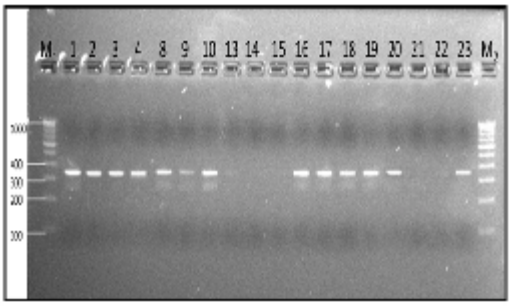

Fig. 1. Amplification of *cap*5K gene obtained from milk from goats with clinical mastitis

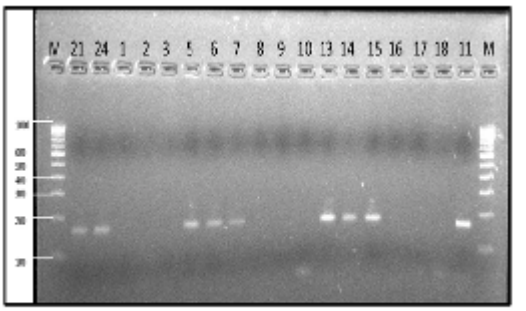

Fig. 2. Amplification of *cap*8K gene obtained from milk from goats with clinical mastitis

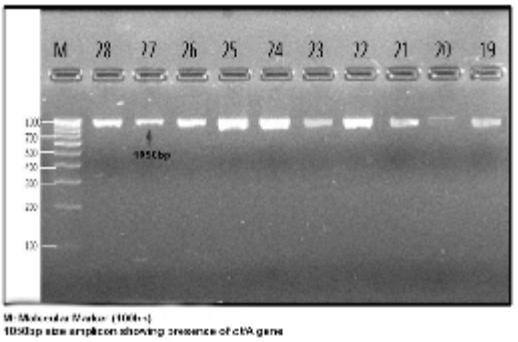

Fig. 3. Amplification of *clf*A gene obtained from milk from goats with clinical mastitis

Momtaz *et al.,* 2010; Proietti *et al.,* 2010; Khichar *et al.,* 2012; Suleiman *et al.,* 2012).

Various workers have experimentally elucidated the antigenic properties and possible role of capsular polysachharides in the generation of vaccines against *S. aureus* infections. The gene cluster for CP5 and CP8 type contains 16 open reading frames (*cap*5A through *cap*5P and *cap*8A through *cap*8P, respectively) the four of which are located in the central region (H - K) and are type specific. In the present study except one isolate (no.22) all other 26 isolates were typable for *cap*5K or *cap*8K gene fragments. Seventeen isolates showed presence of *cap*5Kgene whereas nine showed presence of *cap*8K genes. The amplicon sizes obtained in the present study were almost similar to those obtained by Verdier *et al*. (2007), Upadhyay *et al*. (2010) and Khichar, (2011).

Of the 11 CP types CP5 and CP8 are produced by majority of the animal mastitic isolates (Poutrel *et al.,* 1988; Naidu *et al.,* 1991). Likewise, CP5 and CP8 have been reported to be produced by more than 75% of human isolates (Fatton *et al.,* 1996; Lee *et al.,* 1997). The *cap* genes are highly conserved and the capsular polysaccharides have got limited antigenic specificity (O'Riordan and Lee, 2004). Similarly, other studies have also shown that antibodies to capsular polysaccharides have some protective efficacies for preventing infections in experimental animal (Fatton *et al.,* 1996; Lee *et al.,* 1997). The occurrence of *cap*5K or *cap*8K varies in isolates from different geographical regions (Sordelli *et al.,* 2000; Salasia *et al.,* 2004).

In our study one of the 27 (3.7%) isolates was non-typable for *cap*5 or *cap*8 genes, which is in agreement to the observations of many workers who found a variable percentage of non-typable isolates for these genes. Upadhyay *et al*. (2010) had reported 50% caprine *S. aureus*isolates to be non typable whereas 30% showed presence *cap*5K gene and 20% possessed *cap*8K gene. They did not observe any polymorphism in *cap*5K and *cap*8K genes which is in complete agreement to the present study. However, a very high percentage (86.2%) of non-typable isolates was detected by Sordelli *et al*. (2000) by serotyping. Guidry *et al*.(1998) also recorded 59% of the US isolates and 30% of the isolates from Europe to be CP5 or CP8negative. Similarly, 30% of *S. aureus* from bovine mastitis were reported not to possess CP5 or CP8 by Naidu *et al*.(1991). This finding is almost similar to observations of Khichar, (2011) from the same study area who did not record any of the isolates without *cap* genes in his study involving bovine cattle mastitis isolates. Similarly Singh *et al*.(2011) recorded that only 86.8% (60.7% *cap*5K and 26.1% *cap*8K), 79.0% (56.3% *cap*5K and 22.7% *cap*8K) and 81.1% (22.2% *cap*5K and 18.9% *cap*8K) *S. aureus*isolates from intra-mammary infections in Sahiwal cattle, Karan-Fries cattle and Murrah buffaloes respectively were capsulated.

Khichar, (2011) had also reported capsular typing of cattle mastitic *S. aureus*isolates from the same laboratory and found 92.86% isolates to possess cap5K gene while 7.14% isolates to possess cap8K gene amplicon with no polymorphism in *cap* genes.

In the present study 68.38% isolates were detected with *cap*5K genes and 34.61% with *cap*8K genes among typeable isolates which is in conformity to observations of Upadhyay *et al*.(2010) who recorded that 60% of mastitis isolates from cattle and goats had *cap*5K genes and 20% had*cap*8K gene. Similarly Khichar, (2011) reported 92.86% isolates with presence of *cap*5K gene and 7.14% isolates with presence of *cap*8K gene. Contrarily, Alves *et al*.(2008) recorded CP type 8 with an overall prevalence of 83.1% in ovine and caprine isolates.

Clumping factor A (*clf*A) is a cell surface bound protein in *S. aureus*which is thought to be essential for colonization and establishment of infections. It is known to participate in the infection process by facilitating bacterial binding via soluble or immobilized fibrinogen (Karahan *et al.,* 2011) and has also been suggested as a potential vaccine candidate. Said *et al*.(2010) also suggested *clf*Atyping useful for revealing the clonal nature of mastitis isolate lineage. Risley *et al*.(2007) in a study found that capsular polysaccharide masks *clf*Amediated adherence of *S. aureus*to fibrinogen and platelets and suggested that these findings have important implications in vaccine development.

In the present investigation 25 isolates each produced single amplicon whereas two isolates (Moroni *et al.,* 2005;Aires-de-Sousa *et al.,* 2007) did not produce any PCR amplicon. The observation is in accordance to the finding of Salasia *et al*. (2004), Karahan *et al*. (2011) and

Momtaz *et al*. (2010) who suggested that clumping factor is present in majority of the isolates. The size of the amplicon product obtained in the present study was 1050 bp which is in complete agreement to report of Kalorey *et al*.(2007) who characterized 37 strains of *S. aureus* of bovine milk origin for *clf*Agene and obtained an amplicon of approximately 1042 base pairs in all the isolates. Moroni *et al*.,(2005) characterized *S. aureus* isolated from milk from chronically infected Alpine dairy goats and obtained an amplicon of 1030 bp in all 28 isolates by amplification of *clf*Agene. Similarly, Akineden *et al*.(2001) investigated 103 *S. aureus*isolates from milk of mastitic cows for *clf*Agene and obtained a single amplicon with a size of approximately 1000 bp. Salasia *et al*.(2004) also carried out amplification of *clf*Agene and found a single amplicon with a size of approximately 1000 base pairs.

We did not observe any polymorphism in *clf*Agene which is in accordance with the observations of some other workers from various locations *viz.* Akineden *et al*. (2001) from Germany; Stephan *et al*. (2001) from North-east Switzerland; Salasia *et al*. (2004) from Germany and Momtaz *et al*.(2010) from Iran. Contrarily, polymorphism in *clf*A gene was reported by Fitzgerald *et al*.(2000) who carried out genotyping of 102 Irish and 42 USA *S. aureus*strains of bovine intramammary infection origin and recorded the *clf*Aregion-R amplicons to vary between 220 and 1180 bp in sizes. Similarly, Reinoso *et al*.(2008) on genotyping of *S. aureus*isolates from different sources for *clf*Arevealed an amplicon size of 1000 bp for 40 *S. aureus*whereasfour strains from bovine origin and one strain from food origin had an amplicon of 900 bp size. Likewise, Karahan *et al*.(2011) investigated *S. aureus* isolated from sub-clinical bovine mastitis in Turkey and found that 91.3% of the 92 coagulase positive *S. aureus* isolates carried *clf*Agene in which 68 isolates had *clf*Aamplicon of 1000 bp and remaining 16 isolates had amplicon of 900 bp, the remaining eight isolates did not possess *clf*A gene.

REFERENCES

1. Aarestrup, F.M., Wegener, H.C., Rosdahl, V.T. and Jensen, N.E. Staphylococcal and other bacterial species associated with intramammary infections in Danish dairy herds. *Acta. Vet. Scand.*, 1994; **36**(4): 475-487.
2. Aires-de-Sousa, M., Parente, C.E.S.R., Vieira-da-Motta, O., Bonna, I.C.F., Silva, D.A., de Lencastre, H. Characterization of *Staphylococcus aureus* isolates from Buffalo, Bovine, Ovine and Caprine milk samples collected in Rio de Janeiro State, Brazil. *Appl. Environ. Microb.*,2007; **73**(12): 3845-3849.
3. Akineden, Ö., Annemüller, C., Hassan, A. A., Lämmler, C., Wolter, W. and Zschöck, M. Toxin genes and other characteristics of *Staphylococcusaureus* isolates from milk of cows with mastitis. *Clin. Diagn. Lab. Immun.*, 2001; **8**(5): 959–964.
4. Alves, P.D.D., McCulloch, J.A., Even, S., Le Marechal, C., Thierry, A., Grosset, N., Azevedo, V., Rosa, C.A., Vautor, E. and Le Loir, Y.Molecular characterization of *Staphylococcus aureus* strains isolated from small and large ruminants reveals a host rather than tissue specificity. *Vet. Microbiol.*,2008; **137:** 190-195.
5. Aras, Z., Aydin, I. and Kav, K.Isolation of methicillin-resistant Staphylococus *aureus* from caprine mastitis cases. *Small Ruminant Res*,,2012; **102**(1): 68-73.
6. Ayedin, I., Kav, K. and Celik, H.A. Identification and antimicrobial susceptibility of subclinical mastitis pathogens isolated from hair goats' milk. *J. Ani. Vet. Adv.*,2009; **8**(6): 1086-1090.
7. Balaban, N. and Rasooly, A. Staphylococcal enterotoxins. *I. J. Food Microbiol.*,2000; **61**(1): 1-10.
8. Bergonier, D., Cremoux, R. De., Ropp, R., Lagriffoul, G. and Berthelot, X. Mastitis of dairy small ruminants. *Vet. Res.*,2003; **34:** 689-716.
9. Contreras, A., Sierra, D., Sanchez, A., Correlas, J.C., Marco, J.C., Paape, M.J. and Gonzalo, C. Mastitis in small ruminants. *Small Ruminant Res.*,2007; **68:** 145-153.
10. Cowan, S.T. and Steel, K.J. Cowan and Steel's Mannual for the identification of medical bacteria. Cambridge University Press. Cambridge, 1975.
11. Domanski, P.J., Patel, P.R., Bayer, A.S., Zhang, L., Hall, A.E., Syribeys, P.J., Gorovits, E.L., Bryant, D., Vernachio, J.H., Hutchins, J.T. and Patti, J.M. Characterization of a humanized monoclonal antibody recognizing clumping factor A expressed by *Staphylococcusaureus*. *Infect. Immun.*,2005; **73:** 5229–5232.
12. Fatton, A.I., Sarwar, J., Ortiz, A. and Naso, R. A *Staphylococcusaureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. *Infect. Immun.*,1996; **64:** 1659-1665.

13. Fitzgerald, J.R., Hartigan, P.J., Meaney, W.J. and Smyth, C. J. Molecular population and virulence factor analysis of *Staphylococcus aureus* from bovine intramammary infection. *J. Appl. Microbiol.*,2000; **88** (6): 1028-1037.
14. Guidry, A., Fattom, A., Patel, A., O'Brien, C., Shepherd, S. and Lohuis, J. Serotyping scheme for *Staphylococcusaureus* isolated from cows with mastitis. *Am. J. Vet. Res.*,1998; **59**(12): 1537-1539.
15. Josefsson, E., Hartford, O., O'Brien, L., Patti, J.M. and Foster, T.J. Protection against experimental *Staphylococcusaureus* arthritis by vaccination with clumping factor A, a novel virulence determinant. *J. Infect. Dis.*,2001; **184:** 1572-1580.
16. Kalorey, D. R., Shanmugam, Y., Kurkure, N. V., Chousalkar K. and Barbuddhe, S.B. PCR-based detection of genes encoding virulence determinants in *Staphylococcusaureus* from bovine subclinical mastitis cases. *J. Vet. Sci.*,2007; **8**(2): 151-154.
17. Karahan, M., Nuri Aciki M. and Cetinkaya, B. Investigation of Virulence Genes by PCR in Stapylococcus *aureus* Isolates Originated from Subclinical Bovine Mastitis in Turkey. *Pak. Vet. J.*,2011; **31**(3): 249-253.
18. Khichar, V., Kataria, A. K., Sharma, R. Characterization of *Staphylococcusaureus* of cattle mastitis origin for two virulence–associated genes (*coa* and *spa*). *Comp. Clin. Pathol.*,2012; DOI: 10.1007/s00580-012-1657-5.
19. Khichar, V. Genotypic characterization of *Staphylococcusaureus* from clinical mastitis in cattle in relation to some virulence factors. M.V.Sc. Thesis submitted to RAJUVAS, Bikaner, Rajasthan, 2011.
20. Lee, J. C., Park, J. S., Sara, E. S., Carey, V. and Fattom, A. Protective efficacy of antibodies to the Staphylococcus aureus Type 5 capsular polysaccharide in a modified model of endocarditis in rats. *Infect. Immun.*,1997; **65**(10): 4146-4151.
21. Momtaz, H., Rahimi, E. and Tajbakhsh, E. Detection of some virulence factors in *Staphylococcusaureus* isolated from clinical and subclinical bovine mastitis in Iran. *African J. Biotech.*, 2010; **9** (25): 3753-3758.
22. Moreillon, P., Entenza, J.M., McDevitt, D., Foster, T.J., Francois, P. and Vaudaux, P. Role of *Staphylococcusaureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. *Infect. Immunol.*,1995; **63:** 4738-4743.
23. Moroni, P., Pisoni, G., Vimercati, C., Rinaldi, M., Castiglioni, B., Cremonesi, P. and Boettcher, P. Characterization of *Staphylococcusaureus* isolated from chronically infected dairy goats. *J. Dairy Sci.*,2005; **88:** 3500 -3509.
24. Naidu, A.S., Forsgren,A., Kalfas, S., Watts J.L. and Fournier J.M. Comparison between lactoferrin and subepithelial matrix protein binding in *Staphylococcusaureus* associated with bovine mastitis. *J. Dairy Sci.*,1991; **74:** 3353–3359.
25. Nour El-Din, A. N., Shkreta, L., Talbot, B. G., Diarra, M. S., and Lacasse, P. DNA immunization of dairy cows with the clumping factor A of *Staphylococcusaureus*. *Vaccine*,2006; **24:**1997–2006.
26. O'Riordan, K. and Lee, J.C. *Staphylococcus aureus* capsular polysachharides. *Clin. Microbiol. Rev.*,2004; **17** (1): 218 -234.
27. Patti, J. M. A humanized monoclonal antibody targeting *Staphylococcus aureus*. *Vaccine*, 2004;**22:** S39–S43.
28. Poutrel, B., Boutonnier, A., Sutra, L. and Fournier, J.M. Prevalence of capsular polysachharide type 5 and 8 among *Staphylococcusaureus* isolates from cow, goat and ewe milk. *J. Clin. Microbiol.*,1988; **26:** 38-40.
29. Proietti, P.C., Coppola, G., Bietta, A., Marenzoni, M.L., Hyatt, D.R., Coletti, M. and Passamonti, F. Characterization of genes encoding virulence determinants and toxins in *Staphylococcusaureus* from bovine milk in Central Italy. *J. Vet. Med. Sci.*,2010; **72**(11): 1443-1448.
30. Reinoso, E. B., El-Sayed, A., La¨mmler, C., Bogni, C. and Zschock, M. Genotyping of *Staphylococcusaureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. *Microbiol. Res.*,2008; **163:** 314-322.
31. Risley, A.L., Loughman, A., Bentley, C.C., Foster, T. and Lee, J.C. Capsular polysaccharide masks clumping factor A-mediated adherence of *Staphylococcusaureus* to fibrinogen and platelets. *J. Infect. Dis.*, 2007; **196:** 919-927.
32. Said, K.B., Ismail, J., Campbell, J., Mulvey, M.R., Bourgault, A.M., Messier, S. and Zhao, X. Regional profiling for determination of genotype diversity of mastitis-specific *Staphylococcusaureus* lineage in Canada by use of clumping factor A, pulsed-field gel electrophoresis, and spa typing. *J. Clin. Microbiol.*,2010; **48**(2): 375-386.
33. Salasia, S. I., Khusnan, Z., Lammler, C. and Zschock, M. Comparative studies on pheno- and genotypic properties of *Staphylococcus aureus* isolated from bovine sub-clinical mastitis in central Java in Indonesia and Hesse in

Germany. *J. Vet. Sci.*, 2004; **5**(2): 103-109.

34. Singh, R.S., Kumar, R. and Yadav, B.R. Distribution of pathogenic factors in *Staphylococcusaureus* strains isolated from intra-mammary infections in cattle and buffaloes. *I. J. Biotechnol.*,2011; **10:** 410-416.
35. Sordelli, D. O., Buzzola, F. R., Gomez, M. I., Moore, L. S., Berg,D., Gentilini, E., Catalano, M., Reitz, A. J., Tollersrud, T., Denamiel, G., Jeric, P. and Lee, J. C. Capsule expression by bovine isolates of *Staphylococcusaureus* from Argentina: genetic and epidemiologic analyses. *J. Clin. Microbiol.*,2000;**38**(2): 846-850.
36. Stephan R., AnnemuÈller C., Hassan A. A. and LaÈmmler C. Characterization of enterotoxigenic *Staphylococcusaureus* strains isolated from bovine mastitis in north-east Switzerland. *Vet. Microbiol.*, 2001; **78:** 373-382.
37. Straub, J. A., Hertel, C. and Hammes, W. P. A 23S rRNA target polymerase chain reaction based system for detection of *Staphylococcusaureus* in meat starter cultures and dairy products. *J. Food Protect.*,1999; **62**(10): 1150-1156.
38. Suleiman, A. B., Kwaga, J. K. P., Umoh, V. J., Okolocha, E. C., Muhammed, M., Lammler, C., Shaibu, S. J., Akinden, O. and Weiss, R. Macro-restriction analysis of Staphlyococcus *aureus* isolated from subclinical bovine mastitis in Nigeria. *African J. Microbiol Res.*,2012; **6**(33): 6270-6274.
39. Tuchscherr, L.P.N., Buzzola, F.R., Alvarez, L.P., Lee, J.C. and Sordelli, D.O. Antibodies to Capsular Polysaccharides and Clumping Factor A prevent mastitis and the emergence of unencapsulated and small colony variants of *Staphylococcusaureus* in mice. *Infect. Immun.*,2008; **76**(12): 5738-5744.
40. Upadhyay, A., Kataria, A.K., Sharma, R. and Singh, G. Capsular typing of *Staphylococcusaureus* isolates from cattle and goat mastitis by PCR targeting *cap*5K and *cap*8K genes. *I. J. Ani. Sci.*,2010; **80:** 1062-1065.
41. Verdier, I., Durand, G., Bes, M., Taylor, K.L., Lina, G., Vandenesch, F., Fattom, A.I. and Etienne, J. Identification of the capsular polysaccharides in *Staphylococcusaureus* clinical isolates by pcr and agglutination tests. *J. Clin. Microbiol.*,2007; **45**(3): 725-729.
42. Vernachio, J., Bayer, A. S., Le, T., Chai, Y. L., Prater, B., Schneider, A., Ames, B., Syribeys, P., Robbins, J., and Patti, J. M. Anti-clumping factor A immunoglobulin reduces the duration of methicillin-resistant *Staphylococcusaureus* bacteremia in an experimental model of infective endocarditis. *Antimicrob Agents Chemother.*, 2003; **47:**3400–3406.
43. Wolz, C., Goerke, C., Landmann, R., Zimmerli, W., and Fluckiger, U. Transcription of clumping factor A in attached and unattached *Staphylococcus aureus* in vitro and during device-related infection. *Infect. Immun.*, 2002;**70:** 2758–2762.