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MOLECULAR DETECTION AND GENETIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BOVINE MASTITIC MILK IN MISAN PROVINCE, IRAQ

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Abstract

During the period between March and May 2018, a total of 20 isolates of *Staphylococcus aureus* were detected during processing of 80 bovine milk samples in Misan province, South of Iraq.The bacterial isolation rate of *S. aureus* was 20 (25%) out of 80 positive CMT samples. The amplification of the clumping factor (*clfA*) gene resulted in a single amplicon with a size of approximately 1000 bp in all 20 isolates of current study. The sequence analysis based on (*clfA* gene) showed high identity (99%) between local *S. aureus* isolates (No.1 & No. 2) and NCBI-BLAST *S. aureus* strain BPH2986. The Phylogenetic tree analysis revealed that the isolates of present study with BPH2986 (LR130518.1) were grouped together in the same cluster. This study characterized a high number of isolates (obtained from clinical mastitis cases) and associated isolates showed a higher genetic similarity based on (*clfA*) gene. The higher frequency of clinical mastitis cases reported in this study reflect the incorrect management in the local bovine farms of Misan province. Therefore, it is imperative to strengthen farmers' awareness for using a hygienic procedures in all dairy farms.

Key words : Bovine Mastitis, *Staphylococcus aureus*, *clfA* gene, phylogenetic analysis.

Introduction

Mastitis is the inflammation of the mammary gland mainly due to a bacterial infection and it is characterized by a variety of local and systemic symptoms (Vasudevan *et al*., 2003; Fueyo *et al*., 2005; Smith *et al*., 2005; Kozytska *et al*., 2010). Globally mastitis is the most common infectious disease affecting the dairy cows and remains the most economically important disease of dairy industries around the world (Abd Ellah, 2013). Mastitis is a multi factorial disease and very difficult to control. It results from injury, chemical irritation and infection caused by different bacterial species (Baloch *et al*., 2011). Mastitis is characterized by physical, chemical and bacteriological change in the milk and pathological changes in the glandular tissue of the udder and affects the quality and quantity of milk (Sharma *et al*., 2011).

Staphylococcus aureus is one of the most important pathogens in humans and animals (Haghkhah, 2003). It is among the most common etiologic agents of bovine mastitis and probably represents the most lethal agent because it causes chronic and deep infection in the mammary glands that is extremely difficult to be cured (Barkema *et al*., 2009; Miles *et al*., 1992). It causes big financial economic losses to the dairy industry worldwide, mainly due to reduced milk production and the need to discard contaminated milk (Seegers *et al*., 2003). The virulence of *S. aureus* is generally considered to be multifactorial and due to the combined action of several virulence determinants (Bien *et al*., 2011). *Staphylococcus aureus* is an important food borne pathogen involved in a variety of invasive diseases (Morandi *et al*., 2009).

Clumping factor is an important adhesion protein of *S. aureus* that is governed by *clfA* gene and is thought to be essential for colonization and establishment of infections (Stephan *et al*., 2001). It participates in the infection process by facilitating bacterial binding via soluble or immobilized fibrinogen as fibrinogen plays a significant

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role in platelet thrombus formation (Karahan *et al*., 2011). Clumping factor has been reported to be present in the majority of isolates and the gene *clfA* and has been reported to be important in pathogenicity of bovine mastitis (Salasia *et al*., 2004; Stephan*et et al*., 2001). The antibodies to *clfA* enhanced the protection against infection provided by capsular polysaccharides antibodies (Tuchscherr *et al*., 2008). Therefore, genotyping of isolates is necessary to identify the genetic relatedness of strains and their source of spread and one of the reliable and broad genotyping methodologies is repetitive element sequence based PCR or REP-PCR (Del Vecchio *et al*., 1995). This study was aimed to detect the genetic characterization of *S. aureus* isolated from mastitic cows in Misan province on the basis of *clfA* gene, which has been used firstly for detection of *S. aureus* in mastitic milk in Iraq.

Materials and Methods

Samples collection

A total of 80 bovine milk samples were collected from 4 local dairy farms (20, 20, 25, 15 samples from A, B, C and D farm, respectively) during the period between March and May 2018, in Misan province, South of Iraq, as it's shown in table 3. Milk samples were collected after cleaning the udder from the grimes, bole and dirt by water and drying by a piece of clean cloth then used cotton moistened by alcohol 70% and removing the first flowage of milk, then California Mastitis Test (CMT) was carried out according (Markey *et al*., 2013).

Isolation of *S.aureus*

Positive CMT milk samples were directly inoculated onto mannitol salt agar (MSA) and incubated at 37 ºC for 24 hrs (Quinn *et al*., 1994), then Mannitol fermented colony from primary cultures were purified by subculture onto MSA medium and incubated at 37ºC for 24-48 h. Gram stain slides were investigated according to Barrow and Feltham (2003). And biochemical tests that included catalase and coagulase tube were performed according to Macfaddin (2000).

Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from *Staphylococcus aureus* isolated by using (Presto™ Mini DNA Bacteria Kit, Geneaid, USA) and according to method described by Sreevatsan. The extraction method was don depend on the manufacturing instructions by using gram positive bacteria D N A Protocol extraction method by using "20 mg/ml" lysozyme buffer. One ml of overnight bacterial growth on BHI broth were placed in 1.5ml micro centrifuge tubes and then transferred in centrifuge at 10000rpm for 1 minute. After that, the supernatant was discarded and the bacterial cells pellets were used in genomic DNA extraction and the extraction was done according to company instruction. Then, the extracted DNA was checked by Nano drop spectrophotometer and store in -20^{0}C at freezer until perform PCR assay.

Polymerase chain reaction (PCR)

PCR assay was performed for detection of virulence factor clumping factor A (*clfA* gene) in *S. aureus* isolates according to method described by Monistero *et al*. (2018) by using specific *clfA* forward primers (5'-GGC TTCAGTGCTTGTAGG- 3') and *clfA* reverse primer (5'-TTTTCAGG GTCAATATAAGC-3') (Stephan *et al*., 2001) at PCR product size approximately 1000bp (table 1). These primers were provided by Macrogen Company, Korea. Then PCR master mix was prepared by using (Maxime PCR PreMix kit. iNtRON. Korea). The PCR premix tube contains freeze-dried pellet of Taq DNA polymerase, dNTPs, Tris-HCl (pH 9.0), KCl, $MgCl_2$ and tracking dye and the PCR master mix reaction was prepared according to kit instructions in (Bioneer, Korea) (table 2). The reaction was performed in a thermocycler (Mygene Bioneer.Korea) by set up the following thermocycler conditions (table 3). The PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

DNA sequencing method

DNA sequencing method was performed for study the genetic relationship analysis between local *S. aureus* isolates (*clfA* gene) partial sequence and NCBI-Genbank submitted *S. aureus* isolated (*clfA* gene) by using Phylogenetic tree analysis NCBI-BLAST Homology sequence identity. The PCR product samples were sent to Macrogen Company in Korea to perform the DNA sequencing by AB DNA sequencing system. The phylogenetic analysis was performed based on NCBI-Blast Alignment identification and Maximum Composite Likelihood method by phylogenetic tree UPGMA method (MEGA 7.0 version). Two partial sequences of *clfA* gene reported were submitted to genbank under accession numbers (MK001021 and MK001022).

Results and Discussion

The results of current study showed that all mastitis cases (80), which have been clinically diagnosed appeared positive to CMT. Based on bacterial isolation and biochemical tests the isolation rate of *S. aureus* was 20 (25%) out of 80 positive CMT samples. The highest

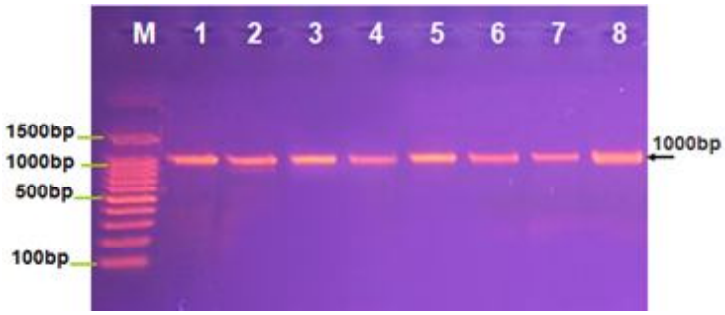

Fig. 1 : Agarose gel electrophoresis image for PCR product in virulence factor clumping factor A (*clfA* gene) *S. aureus* isolates. Where, Lane (M) DNA marker (1500-100bp), Lane (1-8) showed positive *S. aureus* isolates for *clfA* gene at 1000bp PCR product size.

Table 1 : The Mix components of (*clfA)* gene PCR.

Component	Concentration	Amount
Free nuclease water	—	5.5 µl
Green Master Mix	1X	12.5 µl
F Primer	10 pmol/µl	1 µl
R Primer	10 pmol/µl	1 µl
DNA sample	5ng\ µl	5 µl
Total volume	—	25 µl

Table 2 : The PCR program for the (*clfA*) gene amplification.

Step	Temperature (°C)	Time	No. of cycles
Initial denaturation	95°C	5 min	35
Denaturation	95 °C	30 s	
Annealing	57 °C	30 s	
Extension	72 °C	1min	
Final extension	72°C	10 min	

Table 3 : Isolation of *S. aureus* from milk samples collected from four farms located in Miasn province, South of Iraq.

Farms	No.of cow per farm	Samples	Positive *S. aureus* isolates
Farm A	150	20	4(20%)
Farm B	130	20	6 (30%)
Farm C	150	25	7(25%)
Farm D	145	15	3(20%)
Total	575	80(13.9%)	20(25%)

isolation frequency 30% (6/20) and 28% (7/25), were reported in B and C farms, respectively .While the lowest rates of isolation 20% (4/20) and 20% (3/15) were reported in A and D farms, respectively (table 2). These results seem to be similar to the results of earlier studies of Al-Iedani (2016), who noted that, among 143 milk samples, *S. aureus* were detected in 36 (25.17%) samples and also similar ratios were reported by Dehkordi *et al*. (2015), who found the rate of *S. aureus* isolation in dairy products (24.8%) whereas, these result was higher than that was earlier reported (20.59%) in cows suffering from acute mastitis in Baghdad government -Abu-Ghraib zone (Al-Ani, 2009). The difference between our result and result of others may attributed to some factors such as breed difference, different hygiene and management practices followed in each farm, age and parity of the animal and type of the milking (Hammadi and Yousif, 2013). Or due to variation in geographical areas and climatic condition, according to the differences in temperature, humidity, environment and nature of society (International Mastitis Conference, 1995).

Clumping factor A (*clfA*) is necessary in pathogenicity of bovine mastitis, and it is important in colonization because it is a cell surface associated protein of *S. aureus* and this promotes binding of this pathogen to both soluble and immobilized fibrinogen (Stephan *et al*., 2001). The amplification of the clumping factor (*clfA*) gene resulted in a single amplicon with a size of approximately 1000 bp in all 20 isolates of current study (fig. 1), indicating no size polymorphisms of this gene .This result is identical with Momtaz *et al*. (2011), who recorded amplicons of same sizes with no polymorphism. Likewise, Akineden *et al*. (2001) and Salasia *et al*. (2004) reported amplification of the clumping factor gene *clfA* resulting in a single amplicon with a size of approximately 1000 bp from *S. aureus*. But contrary to the result of the previous study, polymorphism was reported 84 (91.3%) out of 92 *S. aureus* isolates showed presence of *clfA* with two different amplicons at the molecular length of approximately 900 bp and 1000 bp (Karahan *et al.*, 2011), another similar study Memon *et al*. (2013) also explained that the size of *clfA* gene amplicon varied from 900 bp to 1000 bp in *S. aureus* isolates associated with bovine mastitis. Even though sequence variances were reported

Table 4 : Homology sequence identity between *S. aureus* isolates virulence factor clumping factor A (*clfA* gene) and NCBI BLAST *S. aureus* isolates.

Staphylococcus aureus	*Genbank accession*	Homology sequence identity	
		NCBI BLAST *Staphylococcus aureus*	Identity %
S.aureus isolate No.1	MK001021	LR130518.1	(99%)
S. aureus isolate ++No.2	MK001022	LR130518.1	(99%)

Fig. 2 : Multiple sequence alignment analysis of virulence factor clumping factor A (*clfA* gene) partial sequence in *S. aureus* isolates with different NCBI-Gen bank *S. aureus* based Clustal W alignment analysis by using (MEGA 7.0, multiple alignment analysis tool). The multiple alignment analysis similarity (*) and differences in virulence factor clumping factor A (*clfA* gene) nucleotide sequence.

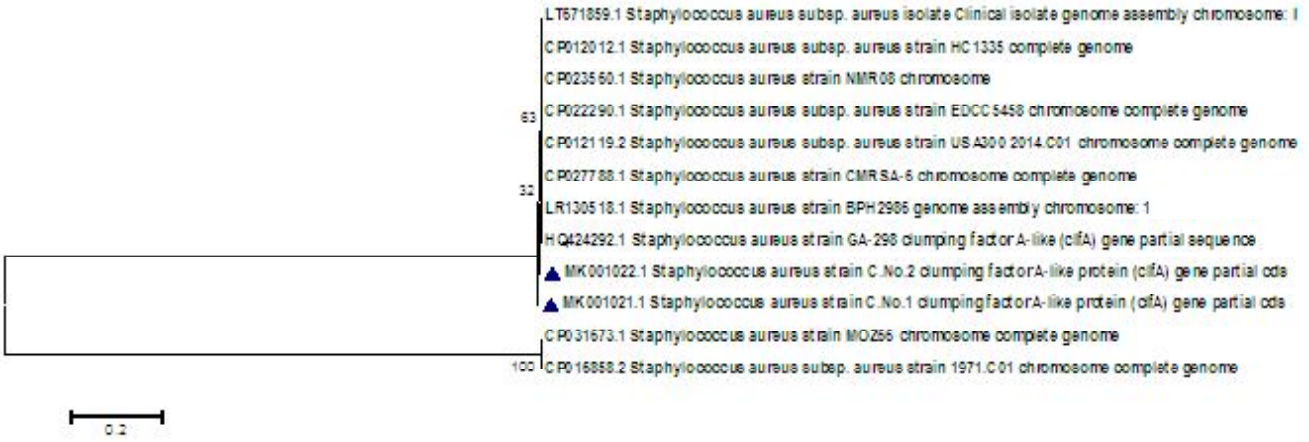

Fig. 3 : Phylogenetic tree analysis based on the partial sequence of virulence factor clumping factor A (*clfA* gene) in *S. aureus* isolates with other reference strains extracted from gene bank data. The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (MEGA 7.0 version).

in previous studies, there is still insufficient knowledge related to the polymorphism in this gene (Karahan *et al.*, 2011).

The sequence analysis based on (*clfA* gene) showed high identity (100%) between local *S. aureus* isolates (No.1 & No.2) and NCBI-BLAST *S. aureus* strain BPH2986 (LR130518.1) (table 4). The Phylogenetic tree analysis revealed that the isolates of present study with BPH2986 (LR130518.1) were grouped together in the same cluster (figs. 2, 3). The similarity among *S. aureus* isolated from bovine mastitis could reflect a long-term persistence into the bovine mammary gland , this indicates that these strains exist among bovine mastitis isolates from other countries (Rabello *et al*., 2007; Sung *et al*., 2008). On other hand, Kate *et al*. (2016) mentioned that *S. aureus* isolates from bovine raw milk sources in Victoria, Australia showed a higher genetic diversity relative to each other and interestingly, the Sa14-004 bovine isolate clustered closer to the caprine and ovine isolates, suggesting it is more distantly related to the other bovine genomes. The results of molecular detection of *S.aureus* from clinical samples (nasal swab, wound, burn sputum and ear swab) were collected from different Iraqi patients showed a type of mutation of Iraqi isolates with the reference strain *S. aureus* strain MN-082 Gen Bank Acc. based on the alignment of partial clumping factor A (*clfA*) gene sequences (Mohammed, 2015). Whereas, no mutations have been detected in the isolates of current study when compared with the reference strain *S. aureus* strain BPH2986 (LR130518.1).

Conclusion

This study characterized a high number of isolates (obtained from clinical mastitis cases) and associated isolates showed higher genetic similarity based on (*clfA*) gene. The higher frequency of clinical mastitis cases reported this study reflect the incorrect management in the local bovine farms of AL-amara city. Therefore, it is imperative to strengthen farmers' awareness for using hygiene procedures in all dairy farms.

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References

AbdEllah, M. R. (2013). Role of Free Radicals and Antioxidants in Mastitis. *Journal of Advanced Veterinary Research*, **3**: 1-7.

Akineden, O. C. Annemuller, A. A. Hassan, C. Lammler, W. Wolter and M. Zschock (2001). Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis.*Clin. Diagn. Lab. Immunol*., **8(5)** : 959-964.

Al-Ani, M. (2009). Immunopathological Study of *Staphylococcus aureus* Isolated from Bovine Mastitis *M.Sc. Thesis.* Vet. Med. College. Baghdad University.

Al-Iedani, A. (2016). Phenotypic study on the capacity of biofilm production in Staphylococcus aureus isolated from bovine subclinical mastitis and their impact on resistance to antimicrobials. *Bas. J. Vet. Res.*, **15(2)**.

Ali, L. Muham, mad G. Arshad, M. Saqib M. and I. J. Hassan (2008). Bacteriology of mastitis in buffaloes in Tehsil sam undri of district Faisalabad, Pakistan. *Pakistan Vet J.*, **28(1)** : 31–33.

Anderson, K. L. and R. L. Lyman (2006). Long-term persistence of speciûc genetic types of mastitis-causing *Staphylococcus aureus* on three dairies. *J. Dairy Sci*., **89** :4551–4556.

Atasever, S. (2012). Estimation of correlation between somatic cell count and coagulation score of bovine milk. *Int J Agric Biol.*, **14**: 315–317.

Baloch, H., R. Rind and D. H. Kalhoro (2011). Study On the Incidence Of Clinical Mastitis In Cattle Caused By Bacterial Species. *Pak. J. Agri., Agril. Engg., Vet. Sci*., **27 (1)**: 83-93.

Barkema, H. W., M. Green, A. J. Bradley and R. N. Zadoks (2009). The role of contagious disease in udder health. *J. Dairy Sci*., **92** : 4717-4729.

Barrow and Feltham (2003). Cowan and Steel's. *Manual for the Identification of Medical Bacteria*. 2nd Ed. Cambridge University press Cambridge, London, New York.USA.

Bhanderi, B. B., A. Roy, M. M. Yadav and C. G. Joshi (2009). PCR based detection of virulence associated genes of *Staphylococcus aureus* from clinical and subclinical bovine mastitis. *R. Vet. J. Ind*., **I&II(5)**: 20-26.

Bien, J., O. Sokolova and P. Bozko (2011). Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. *J. Pathogens*, **601905**. doi:10.4061/2011/ 601905.

Brody, T., A. S. Yavatkar Y., J. Lin, A. Ross, M. Kuzin Y. Kundu Fann and W. F. Odenwald (2008). Horizontal gene transfers link a human MRSA pathogen to contagious bovine mastitis bacteria. *PLos One*, 33074.

Chu, C. C., Y. Yu, Lee and Y. Su (2012). Genetically divergent methicillin resistan *S. aureus* and sec-dependent mastitis of dairy goats in Taiwan. *BMC Vet Res.*, **8** : 39. Con. Brussels, Belgium.

Del, Vecchio V. G., J. M. Petroziello, M. J. Gress F. K., G. P. Mccleskey, H. K. Melcher Crouch and J. R. lupski (1995). Molecular genotyping of methicillin resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive sequence PCR. *J Clin Microbiol*., **33** : 2141-2144.

Fitzgerald, J. R., W. J. Meaney, P. J. Hartigan, C. J. Smyth and V. Kapur (1997). Fine-structure molecular epidemiological analysis of *Staphylococcus aureus* recovered from cows. *Epidemiol Infect*., **119** : 261-269.

Fueyo, J. M., M. C. Mendoza, M. R. Rodicio, J. A. Muniz, M. A. lvarez and M C. Mart (2005). Cytotoxin and pyrogenic toxin superantigen gene profiles of *Staphylococcus aureus* associated with subclinical mastitis in dairy cows and relationship with macrorestriction genomic profiles. *J Clin Microbiol*., **43** : 1278–1284.

Haghkhah, M. (2003). Study of Virulence Factors of *Staphylococcus aureus*. *Ph,D. Thesis,* Faculty of Biomedical and Life Sciences University Glasgow,Scotland, UK.

Hammadi, Kh. M. and A. A.Yousif (2013). Prevalence of clinical and subclinical ovine mastitis caused by *Staphylococcus aureus*.*Al-Anbar J. Vet. Sci.*. **6(1)**.

Hussain, R., M. T. Javed and A. Khan (2012a). Changes in some biochemical parameters and somatic cell counts in the milk of buffalo and cattle suffering from mastitis. *Pak Vet J*., **32** : 418-421.

Hussain, R. M T, A. Javed, F. Khan F, Mahmood and R. Kausar (2012b). Mastitis and associated histo-pathological consequences in the context of udder morphology. *Int J Agric Biol*., **14** : 947-952.

International Mastitis Conference (1995). Bovine mastitis. Document No. 132.

International Mastitis Conference (1995). Bovine mastitis .Document No. 132. *Int. Mast.Con. Brussds, Belgium*.

Iwatsuki, K., O. Yamasaki, S. Morizane and T. Oono (2006). *Staphylococcal cutaneous* infections: invasion, evasion and aggression. *J Dermatol Sci*., **42**: 203–214.

Kalorey, D. R., Y. Shanmugam, N. V. Kurkure, K. K. Chousalkar and S. B. Barbuddhe (2007). PCR-based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. *J. Vet. Sci*., **8 (2)** :151-154.

Karahan, M. Nuri, M. Aciki and B. Cetinkaya (2011). Investigation of virulence genes by PCR in *Stapylococcus aureus* isolates originated from subclinical bovine mastitis in Turkey. *Pak. Vet. J*., **31(3)** : 249-253.

Kate, McMillan Sean, C. Moore, Catherine M, McAuley Narelle, Fegan and M. Fox Edward (2016). Characterization of

Staphylococcus aureus isolates from raw milk sources in Victoria, Australia.*McMillan et al. BMC Microbiology*, **16** : 169.

Kozytska, S., D. Stauss, M. C. Pawlik, S. Hensen, M. Eckart, W. Ziebuhr, W. Witte and K. Ohlsen (2010). Identiûcation of speciûc genes in *Staphylococcus aureus* strains associated with bovine mastitis. *Vet Microbiol*., **145** : 360–365.

Macfaddin, J. F. (2000). *Biochemical tests for identification of medical bacteria*. **3**rd Ed. Lippincott Williams and Wilkins USA.

Markey, B. K., F. C. Leonard, M. Archambault, A. Cullinane and D. Maguire (2013). *Clinical Veterinary Microbiology*. 2nd ed. Mosby Elsevier, pp 105 - 120.

Memon, J., Y. Yang, J. Kashif, M. Yaqoob, R. Buriro, J. Soomro, W. Liping and F. Hongjie (2013). Genotypes, virulence factors and antimicrobial resistance genes of *Staphylococcus aureus* isolated in bovine subclinical mastitis from Eastern China. *Pak. Vet. J*., **33(4)**:486-491.

Miles, H., W. Lesser and P. Sears (1992). The economic implications of bioengineered mastitis control. *J. Dairy Sci*., **75**: 596– 605.

Mohammed, K. M. (2015). Phenotyping and genotyping of some factors effect of *Staphylococcus aureus* adhesive capability. *M.Sc.thesis Genetic Engineering and Biotechnology Institute for Postgraduate Studies University of Baghdad* .105p .

Momtaz, H., E. Tajbakhsh, E. Rahimi and Momeni (2011). Coagulase gene polymorphism of *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis. *Comp. Clin. Pathol*., **20** : 519-522.

Momtaz, H. E, Rahimi and E. Tajbakhsh (2010). Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. *Afr J Biotech*., **9** : 3753-3758.

Monistero, V. Graber, H. U. Pollera, C. Cremonesi, P. Castiglioni and B. Bottini (2018). *Staphylococcus aureus* Isolates from Bovine Mastitis in Eight Countries:*Genotypes, Detection of Genes Encoding Different Toxins and Other Virulence Genes.* Toxins 2018, **10** : 247.

Moon, J. S., LAR, H. M. Lee, E. S. Kang, M. N. Lee, Y. H. Kim, Y. H. Paik, Y. S. Park, Joo and H. C. Koo (2007). Phenotypic and genetic antibiogram of methicillin resistant Staphylococci isolated from bovine mastitis in Korea. *J Dairy Sci*., **90** : 1176-1185.

Morandi, S., M. Brasca, C. Andrighetto, A. Lombardi and R. Lodi (2009). Phenotypic and genotypic characterization of *Staphylococcus aureus* strains from Italian dairy products. *International Journal of Microbiology*, 1–7.[PMC free article][PubMed].

Moroni, P., G. Pisoni, C. Vimercati, M. Rinaldi, B. Castiglioni, P. Cremonesi and P. Boettcher (2005). Characterization of *Staphylococcus aureus* isolation from chronically infected dairy goats. *J. Dairy Sci*., **88**: 3500 –3509.

Nathawat, P., T. Bhati, S. K. Sharma, N. Mohammed and A. K. Kataria (2013). Prevalence of *Staphylococcus aureus* in lactating goats with clinical mastitis and their antibiogram studies. *Abah Int. J. Bioflux*, **5(1)** : 32-37.

Quinn, P. J., M. E. Carter, B. K. Markey and G. R. Carter (1994). Clinical Veterinary Microbiology. Wolfe Publishing, Mosby-Year Book Europe Ltd. Lynton House, 7-12. Tavistock Square, London WCH 9LB, England.

Rabello, R. F., B. M. Moreira, R. M. Lopes, L. M. Teixeira, L. W. Riley and A. C. Castro (2007). Multilocus sequence typing of *Staphylococcus aureus* isolates recovered from cows with mastitis in Brazilian dairy herds. *J Med Microbiol*., **56** : 1505–1511.

Radostits, OM. CC, Gay. DC, Blood and K. W. Hinchcliff (2003). *Veterinary Medicine*. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th ed., Saunders, Philadelphia, USA.

Salasia, SIO. Z, Khusnan C, Lammler and M. Zschock (2004). Comparative studies on phenotypic and genotypic propertie of *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. *J. Vet.Sci*., **5(2)** : 103-109.

Salem-Bekhit, M. M., M. M. Muharram, I. M. Alhosiny and ME. SY. Hashim (2010). Molecular detection of genes encoding virulence determinants in *Staphylococcus aureus* strains isolated from bovine mastitis. *J. Appl. Sci. Res*., **6(2)**: 121-128.

Seegers, H., C. Fourichon and F. Beaudeau (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res*., **34** : 475-491.

Seki, K. J., H. K. Sakurada, M. Seong, H. Murai, H. Tachi, Ishii and S. Masuda (1998). Occurrence of coagulase serotype among *Staphylococcus aureus* strains isolated from healthy individuals-special reference to correlation with size of protein-A gene. *Microbiol Immunol*., **42**: 407–409.

Sharma, N., N. K. Singh and M. S. Bhadwal (2011). Relationship of somatic cell count and mastitis. Division of veterinary clinical medicine. *Asian- Aust. J. Animal. Sci*., **24 (3)**: 429 - 438.

Smith, E. M., L. E. Green, G. F. Medley, H. E. Bird, L. K. Fox, Y. H. Schukken, J. V. Kruze, A. J. Bradley, R. N. Zadoks and C. G. Dowson (2005). Multilocus sequence typing of intercontinental bovine *Staphylococcus aureus* isolates. *J Clin Microbiol*., **43** : 4737-4743.

Smith, E. M., L. E. Green, G. F. Medley, H. E. Bird and C. G. Dowson (2005). Multilocus Sequence Typing of *Staphylococcus aureus* Isolated from High-Somatic-Cell-Count Cows and the Environment of an Organic Dairy Farm in the United Kingdom. *Journal of Clinical Microbiology*, **43** : 4731-4736.

Stephan, R., C. Annemuller, A. Hassan and C. Lammler (2001). Characterization of enterotoxigenic *Staphylococcus*

aureus strains isolated from bovine mastitis in north-east Switzerland. *Vet. Microbiol*., **78** : 373-382.

Straub, J. A. C., Hertel and W. P. Hammes (1999). A 23S rRNA-targeted polymerase chain reaction-based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. *J. Food Prot*., **62** : 1150-1156.

Sung, J. M., D. H. Lloyd and J. A. Lindsay (2008). *Staphylococcus aureus* host speciûcity: comparative genomics of human versus animal isolates by multistrain microarray. *Microbiology*, **154** : 1949–1959.

Tuchscherr, L. P. N., F. R. Buzzola, L. P. Alvarez, J. C. Lee and D. O. Sordelli (2008). Antibodies to capsular polysaccharide and Clumping factor A prevent mastitis and the emergence of un encapsulated and small-colony variants of *Staphylococcus aureus* in mice. *Infect. Immunol*., **76(12)** : 5738-5744. .

Vasudevan, P., M. K. Nair, M. Annamalai T. and K. S. Venkitanarayanan (2003). Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for bioûlm formation. *Vet Microbiol*., **92** : 179–185.

Zadoks, R. van Leeuwen, W. Barkema, H. Sampimon, O. Verbrugh, H. Schukken Y. and A. van Belkum (2000). Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. *J. Clin. Microbiol*., **38** : 1931–1939.