U. of K. J. Vet. Med. Anim. Prod., Vol.6, Issue 2 (2015) p 118-126

ISSN: 243456

UNIVERSITY OF KHARTOUM
Journal of Veterinary Medicine and Animal Production

Staphylococcus aureus mastitis: Influence on Milk Composition and Susceptibility to Antibiotics

Rasha T. A. Mohammed and Ibtisam, E. M. El Zubeir[3]

of Dairy Production, Faculty of Animal Production, University of Khartoum, P. O. Pox 321, Khartoum, Sudan, Ibtisammohamed@hotmail.com

Abstract

The present study was conducted with the aim of investigating the occurrence of *Staphlococcus aureus* mastitis in dairy farms in Khartoum State and the chemical composition of *S. aureus* mastitis milk. Bacteriological analysis and chemical composition was determined with milk analyser Lactoscan. Sixty mastitic milk samples were collected, from which 22 samples were *S. aureus*. Antimicrobial sensitivity tests, using 8 different antibiotics, were performed against the isolates. About 91% of the isolates were sensitive to chloramphenicol and gentamicin, 86.4% to ciprofloxacin, 77.3% to penicillin G, 64% to augmentin and 63.6% to vancomycin. However, 59% of isolates were resistant to fusidic acid and 54.5% to erythromycin. Chemical analysis of mastitic milk Revealed that the mean value of fat, protein, lactose and solids non fat were 3.00±1.4%, (range 1.6%- 4.1%), 3.6±0.4%, (range 2.9%- 4.3%), 4.8±0.8%, (range 3.8%- 5.6%) and 9.1±0.9% (range 7.5%- 10.5%), respectively. It is concluded that mastitis caused by *S. aureus* might decrease of the nutritive value of milk through reducing its chemical constituent level. Therefore, applying proper mastitis treatment and control measurement is highly recommended.

المستخلص

المستخلص :الهدف من هذه الدراسة هو إستقصاء حدوث إلتهاب الضرع بالعنقودية الذهبية في مزارع الألبان بولاية الخرطوم و معرفة المكونات الكيميائية للبن المصاب بالعنقودية الذهبية. أجريت التحاليل البكتيريولوجية بالطرق التقليدية ودرست المكونات الكيميائية للبن بجهاز مسح اللبن اللاكتوسكان. جمعت 60عينة التهاب ضرع، 22 منها مصابة بالعنقودية الذهبية. تضمنت التحاليل إختبار الحساسية لثمانية مضادات حيوية مختلفة لمعرفة حساسية العزلات. أوضحت النتائج أن 91% من العينات حساسه للكلورامفينيكول و الجنتمايسين، و86.4% للسبروفلوكساسين, و77.3% للبنسلين المائي، و64% للأوغمانتين، و63.6% للفانكومايسين، على أن 59% من العزلات كانت مقاومة للفيوسيدك أسيد و54.5% للأرثرومايسين. تضمنت الدراسة التحاليل الكيميائية نسبة البروتين و الدهن واللاكتوز والجوامد غير الدهنية في اللبن المصاب بإلتهاب الضرع . وكان متوسط القيمه للدهن والبروتين و اللاكتوزوالجوامد غير الدهنيه 3 ± 4.1) %مدي من 1.6% الي 4.1%) و 3.6)%±0.4مدي 2.9% -4.3%) و4.8± 0.8% (مدي 3.8% - 5.6 %) و 9.1± 0.9 % (مدي - 7.5% 10.5%)على التوالى. خلصت الدراسة إلى أن التهاب الضرع المسبب بالعنقودية الذهبية ينتج عنه انخفاضاً فى القيمة الغذائية للبن من خلال نقصان مكوناته , لذا توصي الدراسة بتطبيق برنامج معالجة سليمة لالتهاب الضرع خاصة المسبب بالعنقودية الذهبية .

Introduction

Mastitis is a multi-factorial disease; its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms, and presence of environmental risk factors, as well as interactions between these factors (Suriyasathaporn *et al.,* 2000 and Oviedo-Boyso *et al.,* 2007). These infections are most often due to *Staphylococcus aureus* and Streptococci (Harmon, 1994, Djabri *et al.,* 2002; Contreras *et al*., 2003 and El Zubeir *et al.,* 2006). *Staphylococcus aureus* is one of the most important worldwide causes of mastitis in cattle (El Zubeir *et al.,* 2006; Schukken *et al*., 2009; Tenhagen *et al*., 2009; Abera *et al*., 2010; Hamid *et al.* 2012 and El Zubeir *et al*., 2012). Moreover, Schukken *et al*. (2009) reported that 3% of all animals are infected with *S. aureus*. However, *S. aureus* represents 10 to 12% of all clinical mastitis infections (Tenhagen *et al*., 2009).

Preventive and control measures have to be taken on farm with *S. aureus* mastitis problems and the treatment of *S. aureus* mastitis is associated with poor success leading to a relatively high culling rate (Mohamed *et al.,* 1995 and Smole *et al.,* 1998).

Antimicrobial therapy is a primary tool for controlling staphylococcal mastitis and antimicrobial susceptibility tests help guide the veterinarian in selecting the most appropriate antimicrobial agent for treatment of intramammary infection caused by *S. aureus* (Watts *et al.,* 1995). However, the wide spread use of β. lactamse antibiotics has led to a dramatic increase of *S. aureus* resistant to these antibiotics (Watts and Salmon, 1997, and El Zubeir *et al.,* 2006). In Sudan, Mohamed *et al*. (1993); El Zubeir *et al*. (2006) and Hamid *et al.* 2012 reported the resistance of *S. aureus* to different antibiotics.

Staphylococcus aureus has been reported to frequently show multiple antimicrobial resistance patterns (Enright, 2003 and Normmano *et al*., 2001). The number of *S. aureus* strains that exhibits antimicrobial-resistance properties has increased, together with the potential risk of transmitting the same properties to the human microflora via foods or inducing infections hard to be treated (Normmano *et al*., 2006). Moreover, the occurrence of tetracycline resistance among *S. aureus* was reported from mastitis cases (El Zubeir and El Owni, 2006, Hamid *et al.* 2012 and El Zubeir *et al*., 2012).

Mastitis caused several changes in the composition of milk (Mohamed *et al.,* 1997; Mohammed *et al.,* 1998; Mohammed *et al.,* 1999 and Hamid *et al.,* 2012). The degree of tissue damage and change of the permeability of blood vessels vary according to the concentration and virulence of the causative agents (Munro *et al.,* 1984 and Mohamed *et al.,* 1997). *Staphylococcus aureus* and other organisms cause more severe tissue damage of secretary cells (Kitchen, 1981 and Mohamed *et al.,* 1997). Moreover Middleton *et al.* (2002) reported that *S. aureus* caused a significant ($P<0.01$) decrease of somatic cell counts in the infected quarter compared with the unifected quarter in the same cow.

The objectives of this study are isolation and enumeration of *S. aureus* from mastitic cows and

determination of its influence on the chemical composition of milk. It's also aimed to determine the sensitivity of the isolates to commonly used antibiotics

Materials and methods

Investigated animals and sample collection

Mastitic dairy cattle (cross breeds) were chosen for this study from Khartoum State. Mastitis milk samples (n=60) were collected from Shambat (n=10), Hilat Kuku (n=16), Alkadaro (n=8), Alseleit (n=5) Elrudwan project (n=21).

The udder was first cleaned with soap and water then dried with clean towels. The teats and orifices were swabbed with 70% alcohol and dried with clean cloth. Approximately 100 ml of milk samples from infected quarters were taken into sterile universal bottles, which were labeled and stored immediately into ice boxes (4- 0°C) and transferred to the laboratory.

Microbiological examination

Sterilization

Medium used in this study including nutrient agar (Oxoid CM3), blood agar base (Oxoid CM55), mannitol salt agar (Oxoid, England), nutrient broth (Hi Media, Mumbai) and peptone water were sterilized by autoclaving at 15 pounds pressure for 15 minutes at 121 °C. The sugars media were sterilized by autoclaving at 115 °C for 5 minutes. Glassware such as Petri dishes, test tubes, pipettes and flasks were sterilized in a hot oven at 160°C for one hour (Barrow and Feltham, 2003).

Isolation of *Staphylococcus aureus*

The milk samples were activated by incubation for 12 hours at 37°C in Nutrient broth before under taking the bacteriological analysis. Then the milk samples were examined for the presence of *Staphylococcus aureus* using mannitol salt agar and blood agar base (Marshall, 1992). The purified isolates were kept in Nutrient agar (for further characterization.

Purification of bacterial isolates

A part of typical well isolated colony was picked by sterile wire loop and streaked on the surface of a Petri dish containing a fresh nutrient agar medium. Sub-culturing for each organism was repeated until a pure colony representing the organism was obtained.

The Gram stain was used to check the culture purity. The culture was streaked onto the surface of nutrient agar in universal bottle and incubated at 37°C. The bottles were then stored at 5°C till used. The identification of the purified colonies was carried out according to Barrow and Feltham (2005). The purified isolates were subjected to primary tests (morphological appearance, Gram stain, catalase test, oxidase test, motility test and oxidation- fermentation test) and Secondary confirmatory test (fermentation of sugars, methyl red (MR), Voges- Proskauer (VP) and tube coagulase test).

Sensitivity test

The organisms to be tested were inoculated in nutrient broth diluents and after incubation at 37°C for one hour; they were transferred to the antibiotic plate (Muller & Hinton) as was described by El Zubeir *et al.* (2006). It had the

following antibiotics: penicillin G, gentamicin, augmentin, ciprofloxacin, erythromycin, fusidic acid, chloramphenicol, vancomycin. The plates were incubated for 24 hours and the result was recorded as sensitive or resistant.

Chemical analysis of milk constituents

The milk constituents (fat, protein, lactose and solids not fat) were determined by milk analyzer Lactoscan Milk Analyzer (Milk tronic LTD, Europe) according to the manufacturer's instructions.

The procedure of the analysis was described previously by Abd Elrahman *et al* (2009). The milk samples were mixed gently to avoid any air enclosure in the milk. Then 25 ml of the samples were taken in the sample-holder with the analyzer in the recess position. Then when the starting button was activated, the analyzer sucked the milk, made the measurement, returned the milk in the sample-holder and the digital indicator (IED display) showed the specified results.

Statistical analysis

The data were arranged into computer coding format to facilitate the statistical analysis. Then the data were analyzed by SPSS 19 (Statistical Packing for Social Science) for knowing is there any difference between the five farms

Results

Occurrence of *S. aureus* causing mastitis in dairy herds

S. aureus bacteria (n= 22) was isolated from 60 mastitic milk samples in Khartoum State. The occurrence of *S. aureus* isolates was as follows, Shambat: 1 (10%), Hilat Kuku: 7 (43.8%), Alseleit: 0 (0%), Alkadaro: 4 (50%) and Alrudwan: 10 (47.6%) as shown in Table 1.

The fat content of milk samples infected by *S. aureus* mastitis revealed minimum value of 0.6% in the farms located at Alrudwan dairy camp and a maximum value of 4.8% in the farms located at Shambat. The lower value for the protein of *S. aureus* mastitic milk was found in the samples collected from Hilat Kuku. Whereas the higher value was found from the samples collected from Shambat (Table 2). The mastitic samples collected from Alseleit revealed a lower value for lactose compared to the samples collected from other locations. Moreover, those samples also showed the lowest values for solids not fat (Table 2).

Antibiotic sensitivity of *S. aureus* causing mastitis in dairy herds

Figure 1 presents the sensitivity to antibiotics of the isolated *Staphylococcus aureus* from mastitic milk samples. It is clear that there was high sensitivity of *S. aureus* isolates towards the different antibiotics used. Chloramphenicol (91%), and gentamicin (91%). revealed the higher sensitivity followed by ciprofloxacin (86.4%) and penicillin G (77.3%). However, the sensitivity was less frequent towards augmentin (64%) vancomicin (63.6%), erthromycin (45.5%) and fusidic acid (41%).

Chemical composition of *S. aureus* mastitic milk

Table 1: Occurrence of *S. aureus* mastitis in different dairy farms in Khartoum State

Locations	*S. aureus*	Other organisms	No of samples	Percentage
Shambat	1	9	10	10
Hilat Kuku	7	9	16	43.8
Alseleit	0	5	5	0
Alkadaro	4	4	8	50
Alrudwan	10	11	21	47.6
Total	22	38	60	36.7

Table 2: Mean, minimum and maximum values of chemical composition of mastitic milk collected from different dairy farms

Items	Locations	Mean ± SD	Minimum	Maximum
Fat	**Shambat**	3.3 ± 0.9	2.3	4.8
	Hilat Kuku	1.9 ± 1.1	0.9	4.2
	Alseleit	5.3 ± 3.2	2.4	4.7
	Alkadaro	2.2 ± 0.4	1.6	2.4
	Alrudwan	2.1 ± 1.3	0.6	4.6
	Overall mean	3.00 ± 1.4	1.6	4.1
Protein	**Shambat**	4.0 ± 0.7	3.2	4.9
	Hilat Kuku	3.4 ± 0.3	2.6	4.1
	Alseleit	3.1 ± 0.2	2.8	3.3
	Alkadaro	3.8 ± 0.4	3.5	4.5
	Alrudwan	3.6 ± 0.4	2.6	4.5
	Overall mean	3.6 ± 0.4	2.9	4.3
Lactose	**Shambat**	5.2 ± 0.5	4.3	5.8
	Hilat Kuku	4.8 ± 0.5	3.8	5.7
	Alseleit	3.6 ± 1.8	2.4	4.5
	Alkadaro	5.4 ± 0.6	4.9	6.4
	Alrudwan	4.9 ± 0.5	3.8	5.8
	Total	4.8 ± 0.8	3.8	5.6
SNF	**Shambat**	9.7 ± 0.9	8.1	10.9
	Hilat Kuku	8.8 ± 0.9	6.9	10.6
	Alseleit	7.9 ± 0.7	6.8	8.4
	Alkadaro	9.9 ± 1.2	9.0	11.8
	Alrudwan	9.2 ± 0.8	6.9	10.7
	Overall mean	9.1 ± 0.9	7.5	10.5

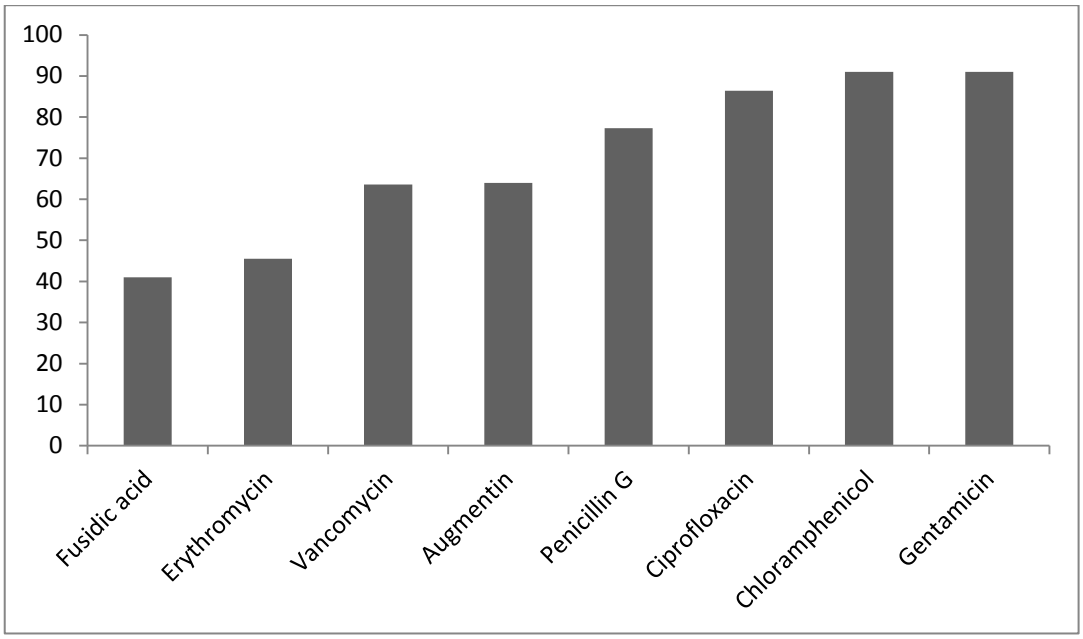

Figure 1: Antibiotic sensitivity tests for *Staphylococcus aureus* isolated from mastitic milk of dairy cows in Khartoum State

Discussion

The present study has shown the isolation of *S. aureus* from different farms located in 5 locations in Khartoum State. The variation reported in the occurrence of *S. aureus* in the different farms supported Berry *et al.* (2007) who reported variations in prevalence of mastitis between different cattle breeds. In the present study, the isolation of *S. aureus* from infected cows (36.7%) indicated that *S. aureus* is one of important pathogens causing mastitis. This result was in line with Mohamed *et al.* (1997); Hameed *et al.* (2006); Abera *et al.* (2010) and Hamid *et al.* (2012). The high occurrence of *S. aureus* mastitis was attributed to inadequate hygienic condition of the dairy environment in Khartoum State (El Zubeir *et al.*, 2006 and El Zubeir and Ahmed, 2007). Similarly, Roberson *et al.* (1994) found that the mean prevalence of *S. aureus* intramammary infection in high prevalence herd (>10%) was 30%, whereas the mean prevalence of *S. aureus* intramammary infection in a low prevalence (< 5%) herds was 2%. In Sudan, Mohamed *et al.* (1997) found 23% of mastitis cases in Friesian herd were caused by *S. aureus*. The higher occurrence reported than those found by Schukken *et al.* (2009) and Tenhagen *et al.* (2009) could be due to the variation in the geographical locations investigated. The high rate of isolation of *S. aureus* may be attributed to the fact that the principal reservoirs of *S. aureus* are the skin of the udder and milk of the infected gland as was reported previously by El Zubeir *et al.* (2006). In addition, *S. aureus* has the capacity to penetrate into the tissue, producing deep-seated

foci protected by a tissue barrier (Ranjan *et al.* 2010).

The present study also investigated susceptibility of the isolates to eight antibiotics. Chloramphenicol and gentamicin were found to be more effective antibiotic (91%) among all tested antibiotics against the *S. aureus* isolated, followed by ciprofloxacin (86.4%). This finding supported Mahantesh and Passaga (2011) and Hamid *et al.* (2012). Chloramphenicol was found as the best antimicrobial agent against *S. aureus*, this result is in line with that reported by Singh and Boxi (1982).

The result showed significant variations in fat, protein, lactose and solids non fat of milk from mastititc cows in the different farms. Mastitis is well known to influence the chemical composition of milk (Mohamed *et al.*, 1997; Mohamed *et al*., 1999; Middleton, 2002 and Hamid *et al.*, 2012). Moreover, *Staphylococcus aureus* cause severe tissue damage that resulted in secretion of cells into milk (Kitchen, 1981 and Petersson-Wolfe *et al*., 2010).

The fat content from mastitis milk was decreased and this may be due to the increase of lipolysis, this result agreed with Murphy *et al* (1989) and Hamid *et al*. (2012). It may also be due to the damage effects of *S. aureus* on secretory tissue (Kitchen *et al.,* 1984). The higher value of fat content of mastitic milk reported at Alseleit could be due to the reason some cows at colostrum stage. The variation of total protein in this study (Table 3) could be due to as explained by Jones (2006) that the milk proteins breakdown from animals with clinical or subclinical mastitis is due to the presence of proteolytic activity by more than 2- fold during mastitis. Also plasmin and enzymes derived from somatic cells can cause extensive damage to casein in the udder before milk removal (Schallibaum, 2001). However, Kitchen (1981) reported that total protein concentration in milk does not change a great deal with increasing the level of infection in mammary gland. The decrease of lactose values of mastitic milk collected during the present study supported the finding of Mohamed *et al.* (1999). Moreover, Mohamed *et al*. (1997) reported lower lactose content in the staphylococcal infected milk of Friesian cows.

Conclusion

This study showed high occurrence of *S. aureus* among dairy cows in different locations of Khartoum State. The udder inflammation or mastitis caused by *S. aureus* has a negative effect on the milk composition. Hence more efforts are needed to improve general udder health in dairy herds especially by application of treatment using suitable drug as in this study some antimicrobial agents revealed higher activity against *S. aureus* strains tested. Therefore, more attentions should be directed towards a good management practices.

References

Abd Elrahman, Samia, M. A.; Said Ahmed, A. M. M.; El Zubeir, Ibtisam, E.M.; El Owni, O. A. O. and Ahmed, M. K. A. (2009). Microbiological and physicochemical properties of raw milk used for processing milk in Blue Nile Dairy Company (Sudan). Australian Journal of Basic and Applied Sciences, 3 (4): 3433 – 3437.

Abera, M.; Demie, B.; Aragaw, K.; Regassa, F. and Regassa, A. (2010). Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance

patterns in Adama town, Ethiopia. Journal of Veterinary Medicine and Animal Health, 2: 29-34.

Barrow, G. I. and Feltham, R. K. A. (2003). Manual for the identification of Medical Bacteria. 3rd, Cambridge, University press London.

Berry, D.P.; Lee, J.M.; Macdonald, K.A.; Stafford, K.; Matthews, L. and Roche, J.R. (2007). Associations among body condition score, body weight, somatic cell count, and clinical mastitis in seasonally calving dairy cattle. Journal of Dairy Science, 90: 637- 648.

Contreras, A.; Luengo, C.; Sanchez, A. and Corrales, J. C. (2003). The role of intramammary pathogens in dairy goats. Livestock Production Science, 79: 273-283.

Djabri, B.; Bareille, N.; Beaudeau, F. and Seegers, H. (2002). Quarter milk somatic cell count in infected dairy cows: Ameta-analysis. Veterinary Research, 33(4): 335-357.

El Zubeir, I. E. M. and Ahmed, M. I. A. (2007). The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. Research Journal of Microbiology, 2(12): 988-991.

El Zubeir, I. E. M.; Kutzer, P. and El Owni O.A.O. (2006). Frequencies and antibiotic susceptibility patterns of bacteria causing mastitis among cows and their environment in Khartoum State. Research Journal of Microbiology, 1: 101 – 109.

El Zubeir, I. E. M. and El Owni, O. A. O. (2006). Seasonal variation of incidences and etiological agents of bovine mastitis in Friesian cattle in Sudan. Research Journal of Animal and Veterinary Sciences, 1: 25-29.

El Zubeir, I. E. M.; Kiessling, S.; Kutzer, P.: Wieler, L.H. and El Owni, O.A.O. (2012). Characterization of tetracycline-resistant *Staphylocoocus aureus* isolated from bovine mastitis in Khartoum state. University of Khartoum Journal of Veterinary Medicine and Animal Production, 3 (2): 53- 64.

Enright, M.C (2003). The evolution of resistant pathogen: The case of MRSA. Current Opinions in Pharmacology, 3: 474–479.

Hameed, K.G.A.; Sender, G. and Korwin-Kossakowska, A. (2006). Public health hazard due to mastitis in dairy cows. Animal Science Papers and Reports, 25: 73- 85.

Hamid, I. M. B.; Shuiep, E. S.; El Zubeir, I. E. M.; Saad, A. Z. and El Owni, O. A. O. (2012). Influence of *Staphylococcus aureus* mastitis on milk composition of different dairy breeds of cattle in Khartoum State, Sudan. World's Veterinary Journal, 2(2): 13-16.

Harmon, R. J. (1994). Physiology of mastitis and factor affecting somatic cell counts. Journal of Dairy Science, 77: 2103- 2112.

Jones, G. M. (2006). Understanding the Basics of Mastitis. Virginia Cooperative Extension. Publication No. 404-233. Virginia State University, USA.

Kitchen, B. J. (1981). Review of the progress of dairy science: Bovine mastitis, milk composition changes and related diagnostic tests. Journal of Dairy Research, 48: 167- 188.

Kitchen, B. J. C.; Sergkwee, W.; Middleton G. and Andres, R. J. (1984). Relationship between the levels of NAGase in bovine milk and the presense of mastitis pathogens. Journal of Dairy Research, 51(1): 11- 16

Marshall, T. R. (1992). Standard Methods for the Examination of Dairy Products. 16th ed. American Public Health Association, Washington, D.C. USA.

Mahantesh, M. K. and Basappa, B. K. (2011). Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. Advances in Applied Science Research, 2 (6): 229-235.

Middleton, J. R.; Fox, L. K. and Tyler, J. W. (2002). Influence of *Staphylococcus aureus* strain- type on mammmary quarter milk somatic cell count and N-Acetyl- B- D- glucosamindase activity in cattle from eight dairies. Journal of Dairy Science, 85: 1133 - 1140.

Mohamed, I. E.; El Owni, O. A. O. and Mohamed, G. E. (1993). A study on the incidence and etiology of bovine mastitis in Sudan. The 2nd Sci. Cong. Egyption Society for Cattle Disease, p. 5 -7 Des. 326-338.

Mohamed, I. E.; El Owni, O. A. O. and Mohamed, G. E. (1995). Production losses and economic importance of mastitis among Friesian cattle in Sudan. Sudan Journal of Veterinary Science and Animal Husbandry,34: 59 -63.

Mohamed, I. E.; El Owni, O. A.O. and Mohamed, G. E. (1997). Effect of bacteria causing mastitis on milk constituents. Sudan Journal of Veterinary Science and Animal Husbandry, 36 (1& 2): 125- 136.

Mohamed, I. E.; El Owni, O. A O. and Mohamed, G. E (1998). Effect of season and infection on composition of mastitic milk of Friesian cattle in Sudan. Indian Journal of Animal Sciences, 68(4): 379- 381.

Mohamed, I.E.; El Owni, O.A.O. and Mohamed, G. E, (1999). Effect of mastitis on milk quality

of Friesian cows in Khartoum State. Indian Journal of Animal Sciences, 69: 733- 735.

Munro, G. L.; Grive, P, A. and Kitchen, B.J. (1984). Effect of mastitis on milk yield, milk composition, processing properties and quality of milk products. Australian Journal Dairy Technology, 39 (91): 7 - 16.

Murphy, S. C.; Cranker, K.; Senyk, G. F.; Brabano, D. M.; Seaman, A. I and Galton, D.M. (1989). Influence of bovine mastitis on lipolysis in milk. Journal of Dairy Science, 72 (3): 620-626.

Normanno, G.; Celano, G. V.; Dambrosio, A.; Lassandro, L. and Buonavoglia, C. (2001). Enterotoxins of *Staphylococcus aureus* induce a cytopathic effect in cell lines. Microbiologica, 24: 341–346.

Normanno, G.; La Salandra, A.; Dambrosio, A. and Celano. G. V. (2006). Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. International Journal of Food Microbiology, 115(3): 290–296.

Oviedo-Boyso, J.; Valdez-Alarcon, J. J.; Cajero-Juarez, M.; Ochoa-Zarzosa, A.; Lopez-Meza, J. E.; Bravo-Patino, A. and Baizabal-Aguirre, V. M. (2007). Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. Journal of Infection, 54(4): 399- 409.

Petersson-Wolfe, C. S.; Mullarky, I. K. and Jones, G. M. (2010). *Staphylococcus aureus* mastitis: cause, detection and control. Virginia Cooperative Extension, 404: 229.

Ranjan, R.; Gupta, M. K.; Singh, S. and Kumar, S. (2010). Current trend of drug sensitivity in bovine mastitis, Veterinary World, 3: 17–20.

Roberson, J. R.; Fox, L. X.; Hancock, D. D.; Gay, J. M. and Besser, T. E. (1994). Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms Journal of Dairy Science, 77: 3354- 3364.

Schallibaum, M. (2001). Impact of SCC on the quality of fluid milk and cheese. Proc. 40th Annual Meeting, National Mastitis Council, Madison, USA. Pp: 38-46.

Schukken, Y. H.; Gonzalez, R. N.; Tikofsky, L. L.; Schulte, H. F.; Santisteban, C. G.; Welcome, F. L.; Bennett, G. J.; Zurakowski, M. J. and Zadoks, R. N. (2009). CNS mastitis: Nothing to worry about? Veterinary Microbiology, 134(1-2):9-14

Singh, K. B. and Box, K. K. (1982). Studies on the aetiology and in vitro sensitivity and treatment of subclinical mastitis in milk animals. Indian Veterinary Journal, 59: 191- 198.

Smole, S. C.; Aronson, E.; Durbin, A.; Brecher, S. M. and Arbeit, R. D. (1998). Sensitivity and specificity of an improved rapid latex agglutination tests for identification of methicillin - sensitivity and resistant *Staphylococcus aureus* isolates. Journal of Clinical Microbiology, 36: 1109 - 1112.

Suriyasathaporn, W.; Heuer, C.; Noordhuizen-Stassen, E.N. and Schukken, Y.H. (2000). Hyperketonemia and the impairment of udder defense: A review. Veterinary Research, 31(4): 397-412.

Tenhagen, B. A.; Hansen, I.; Reinecke, A. and Heuwieser, W. (2009). Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. Journal of Dairy Research, 76(2):179-187.

Watts, J. L.; Nickerson, S. C.; Weaver, L. J.; Holmberg, C.; Pankek, J. W. and Fax, L. K. (1995). Antimicrobial susceptibility, of microorganism isolated from the mammary gland of dairy heifers. Journal of Dairy Science, 78: 1637 - 1648.

Watts, J. L. and Salmon, S. A. (1997). Activity of selected antimicrobial agents against strain of *Staphylococcus aureus* isolated from bovine intramammary infection that produce B-lactamase. Journal of Dairy Science, 80: 788 - 791.