MOLECULAR DETECTION OF *STAPHYLOCOCCUS AUREUS* ENTEROTOXINS ISOLATED FROM MASTITIC SHE-CAMELS

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ABSTRACT

A total of 150 raw milk samples were collected from she camels from Aswan, 100 from individual breed in Daraw and 50 from pastoral camels in Shalateen in Egypt. First, clinical examination revealed no cases with clinical mastitis. Second, the collected milk samples were tested by California Mastitis Test and the results revealed that the percent of subclinical mastitis was (39.3%) for total number of milk samples, but the ratio in Daraw (48%) higher than in Shalateen (22%). Third, by conventional culture method, 20 % of samples were positive to Staphylococcus spp. Fourth, Staphylococcal isolates were identified by conventional biochemical test, (46.7%) of these isolate showed positive for coagulase test. Fifth, these coagulase positive isolates submitted for molecular identification targeting 16S rRNA gene (Staphylococcus genus specific), nuc gene (S. aureus species specific) and Staphylococcal enterotoxins genes (SEA, SEB and SEC) by multiplex PCR, (92.9%) positive for 16s rRNA and confirmed as

Staphylococcus spp., (64.3%) positive for nuc gene and confirmed to be S.aureus and (50%) Staphylococcus isolates were enterotoxin-positive to sea, (35.7%) of them for S.aureus strains and (14.3%) for other coagulase positive Staphylococcus spp. Finally, these isolates were submitted for antibiotic sensitivity testing by using various antibiotics and the proportion of isolates resistance to the antibiotics were penicillin G and chloramphenicol (100%), colistin (93%), tetracycline and lincomycin (78.6%), oxalinic acid (71.4%), amoxicillin (50%), neomycin and cefaclor (42.8%), erythromycin (35.7%), ciprofloxacin (28.6%) and gentamycin (21.4%).

INTRODUCTION

Camel's milk can be considered as a good source of minerals, vitamins and characterized by higher ratio of lactoferrin than other dairy milk. Moreover, milk of camel could cover a big part of the daily needs of humans from these nutrients, because camel milk has most the essential nutrients ***(Al-Otaibi and El-Demerdash, 2013)***. In pastoral conditions, milk is always consumed fresh, in the raw state without heat treatments, and this consider as a health hazard to the consumer ***(Al-Majali et al ., 2007)*.**

Mastitis is a complex disease occurring worldwide among the dairy animals either in acute or in chronic form causing heavy economic losses, changes in the hygienic and compositional quality of milk and impairment of the technological properties of milk ***(Wielgosz- Groth and Groth, 2003)*** and decreased reproductive performance ***(Schrick et al., 2001)*.** Additionally, mastitis can be harmful to suckling newborns. Acute and chronic mastitis can be clinically diagnosed by examination of the

udder, the milk, or by both ***(Obied et al., 1996).*** While detection of subclinical mastitis is difficult and depends on various indirect tests as California Mastitis Test and somatic cell count as well as microbial examination ***(IDF, 1987)***.

Bacterial infection are considered the primary cause of mastitis in domestic animals ***(Seifu and Bekele, 2010)***. *S.aureus* is one of the most important pathogen in milk or its products ***(LeLoir et al., 2003)***. *S.aureus* produces a wide variety of toxic proteins such as toxic shock syndrome toxin 1, exfoliative toxins, and enterotoxins (SEs). In addition to the five classical major antigenic types of SEs (SEA, SEB, SEC, SED, and SEE), four additional SEs (SEG, SEH, SEI, SEJ) have been also reported, and their corresponding genes have been described ***(Stephan et al., 2001; Kuzma et al., 2003)***. The staphylococcal enterotoxins are known as being agents of intoxication such as staphylococcal food poisoning in man and they may cause other types of infections ***(Gilmour and Harvey, 1990)***.

It is relevant to consider that the transmission of *S.aureus* is possible either by direct contact with animals or through contaminated food as milk or cheese ***(Vautor et al., 2005)***. Today 90% of human *S.aureus* are penicillin resistant ***(Zinn CE, 1999)***.

The current study aimed to evaluate percent of subclinical mastitis and risk factors associated with it in she-camel, Conventional bacteriological analysis of the mastitis pathogens, Detection of the presence of *nuc* gene of *S.aureus* and investigate the enterotoxins sea, seb and sec genes and application of antimicrobial sensitivity test to evaluate the rate of antibiotic resistance in different *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

1.1. Sampling

A total of 150 raw milk samples were collected from 150 she camels from different localities, 100 from Daraw and 50 from Shalateen. These raw milk samples of she camels were collected during the period from June 2014 to January 2016. Collection, transportation and preparation of samples were based on the guide line described by ***(NMC, 1990)***.

1.2. California Mastitis Test (CMT) according to ***(Schalm and Noorlander, 1957)*****.**

1.3. Culturing method according to ***(Lancett and Bennett, 2001)*****.**

1.4. Coagulase test according to ***(Cookson, 1997)*****.**

1.5. Polymerase chain reaction (PCR).

a. DNA Extraction:

Total DNA extraction was carried out by a rapid boiling method according to ***(Reischl et al., 1994)***.

b. Multiplex PCR assay:

Multiplex PCR assay targeting *16S rRNA* gene (*Staphylococcus* genus specific), *nuc* gene (*S.aureus* species specific), SEA, SEB and SEC was performed. The amplification was performed on thermal cycler by using total volume of 25ul reaction mix contain 5ul of template DNA, 20 pmol of each primer and 1X of PCR mix. Detailed sequences of primers and cycling protocols are depicted in (Tables 1,2). The analysis of PCR products was carried out using 1.5% ethidium bromide stained agarose gel.

c. Agarose gel electrophoresis was carried out according to *(Sambrook and Russel, 2001)*.

Table (1): Primer Sequences used in PCR assay.

Target	Name (strand)	Primer sequence (5 - 3)	Reference
Staphylococcus	16S rRNA- F	5' - GTA GGT GGC AAG CGT TAT CC -3'	Monday and Bohach, (1999)
	16S rRNA-R	5'- CGC ACA TCA GCG TCA G -3'	
S. aureus	*Nuc* –F	5'- GCG ATT GAT GGT GAT ACG GTT-3'	Brakstad *et al.*, (1992)
	Nuc -R	5'- AGC CAA GCC TTG ACG AAC TAA AGC-3'	
SEA	SEA-F	5'- TAAGGAGGTGGTGCCTATGG -3'	Cremonesi *et.al*., (2005)
	SEA-R	5'- CATCGAAACCAGCCAAAGTT-3'	
SEB	SEB-F	5'- TCGCATCAAACTGACAAACG-3'	Johnson *et al.*, (1991)
	SEB-R	5'- GCAGGTACTCTATAAGTGCC-3'	
SEC	SEC-F	5'- ACCAGACCCTATGCCAGATG-3'	Cremonesi *et.al.*, (2005)
	SEC-R	5'- TCCCATTATCAAAGTGGTTTCC-3'	

Table (2): Cycling protocols of PCR assay.

Target	Amplicon size	Cycling program			
		Step	Temp.	Time	No. of cycles
16S rDNA gene	228bp	Initial denaturation	94ºC	4 min	One cycle
Nuc gene	279bp	Denaturation	94ºC	45s	35 cycles
SEA	180bp	Anealing	55ºC	45s	
SEB	478bp	Extention	72ºC	45s	
SEC	371bp	Final extention	72ºC	10min	One cycles

1.6. Antimicrobial susceptibility test was carried out according to the guidelines stipulated by ***(NCCLS, 2001)***.

RESULTS

In this study, 59/150(39.3%) of milk samples collected from she camels were detected with subclinical mastitis, 48/100(48%) from Daraw, 11/50(22%) from Shalateen, depicted in table(3). Table (4) showing the frequency of the isolated *Staphylococci* from the examined milk samples from subclinical mastitis, 30/150(20%) *Staphylococcus* spp isolated from milk samples. Identification of these isolates was performed using phenotypic and genotypic methods. Coagulase test was conducted to 30 positive isolates resulting 14/30(46.7%) coagulase positive *Staphylococci* CPS and 16/30(53.3%) coagulase negative staphylococci CNS, depicted in table (4). The performed multiplex PCR assay for 14 coagulase positive isolates confirmed 13/14 (92.9%) of isolates to be *Staphylococci* by successful amplification of the 228 bp PCR product of the staphylococcal specific *16s rRNA* gene. using the same multiplex PCR, 9/14(64.3%) of isolates were confirmed to be *S.aureus* by successful amplification of 279 bp PCR product of the *S.aureus* specific *nuc* gene, 7/14(50%) of isolates were confirmed to carry sea gene by successful amplification of 180 bp of PCR product of specific sea gene, 5/14(35.7%) of isolates were confirmed to carry sea gene with *S.aureus* and 2/14(14.2) of isolates were confirmed to carry sea gene with other staphylococci, photo (1) showing ethidium bromide stained 1.5% agarose gel electrophoresis of multiplex PCR assay. The antimicrobial sensitivity testing of isolated strains (n=14) to various antimicrobials revealed that (100%)of isolates were resistant to

penicillin G and chloramphenicol followed by colistin (93%), tetracycline and lincomycin (78.6%), oxalinic acid (71.4%), amoxicillin (50%). The isolates show sensitivity to gentamycin (64.3%), neomycin and cefaclor (42.8%). The test show intermediate susceptible result to ciprofloxacin (64.3%) and erythromycin (50%), explained in table (5).

Table (3): prevalence of subclinical mastitis in she camels according to CMT

Samples	NO.	Positive CMT	Score3 +ve	Score2 +ve	Score1 + ve	Negative CMT	Score 0 _ ve	Score trace _ ve
Total no. of tested milk samples	150	59 (39.3%)	39 (26%)	8 (5.3%)	12 (8%)	91 (60.7%)	52 (34.7%)	44 (29.3%)
Daraw samples	100	48 (48%)	33 (33%)	8 (8%)	7 (7%)	52 (52%)	28 (28%)	24 (24%)
Shalaten samples	50	11 (22%)	6 (12%)	0 (0%)	5 (10%)	39 (78%)	24 (48%)	15 (30%)

Table (4): frequency of the isolated *S.aureus* from the examined milk samples from subclinical mastitis

Samples	No. of tested milk samples	Positive Samples	Coagulase Positive *S.aureus*	Coagulase negative staph.	Negative samples
Total no.	150	30 (20%)	14 (9.3%)	16 (10.7%)	120 (80%)
Daraw samples	100	19 (19%)	8 (8 %)	11 (11%)	81 (81%)
Shalateen samples	50	11 (22%)	6 (12%)	5 (10%)	39 (78%)

photo (1): Ethidium bromide stained 1.5% agarose gel electrophoresis of multiplex PCR assay. Lane M:100bp DNA ladder, Lane 1: Positive control contain 3 band (180bp of Sea gene, 228bp of 16S rRNA gene and 279bp of *Nuc* gene), Lanes 2,3,11 and 14: *Staphylococcal aureus* not contain Sea gene Samples, Lanes 4 and 5: Staphylococcal but not *aureus* samples, Lanes 6,8,9,10 and 13: *Staphylococcal aureus* contain Sea gene Samples, Lanes 7 and 12: Staphylococcal but not *aureus* contain Sea gene Samples, Lane 15: Negative samples, Lane 16: Negative control.

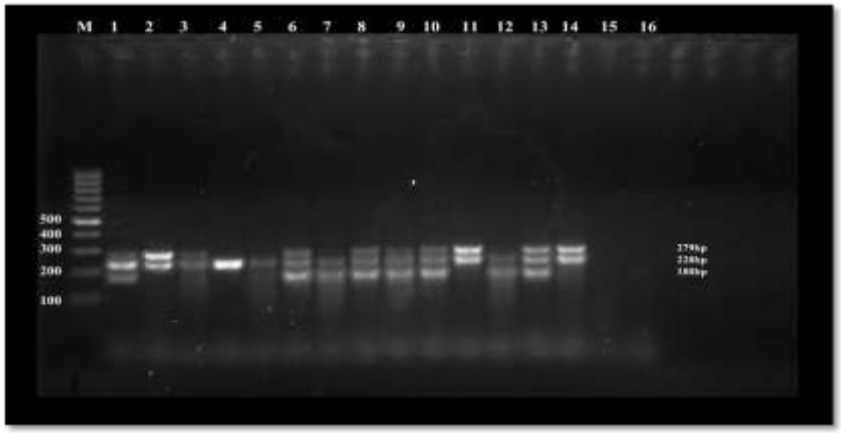

Table (5): Shown Percentage of sensitive, intermediate and resist samples to different antibiotics.

Antibiotics	NO. of sensitive samples	NO. of intermediate samples	NO. of Resist samples
Penicillin G	0 (0%)	0 (0%)	14 (100%)
Neomycin N	6 (42.8%)	2 (14.3%)	6 (42.8%)
Chloramphinicol C	0 (0%)	0 (0%)	14 (100%)
Colistin CT	0 (0%)	1 (7.1%)	13 (93%)
Amoxicillin AX	2 (14.3%)	5 (35.7%)	7 (50%)
Gentamycin CN	9 (64.3%)	2 (14.3%)	3 (21.4%)
Erythromycin E	2 (14.3%)	7 (50%)	5 (35.7%)
Oxalinic acid OA	4 (28.6%)	0 (0%)	10 (71.4%)
Cefaclor CEC	6 (42.8%)	2 (14.3%)	6 (42.8%)
Tetracycline TE	0 (0%)	3 (21.3%)	11 (78.6%)
Lincomycin L	2 (14.3%)	1 (7.1%)	11 (78.6%)
Ciprafloxacin CIP	1 (7.1%)	9 (64.3%)	4 (28.6%)

DISCUSSION

In present study, the clinical examination of 150 she camels, 100 from Daraw and 50 from Shalateen revealed that all examined animals have no clinical signs related to mastitis .

According to ***(Shearer and Herris, 2003)*** subclinical mastitis is important due to the fact that it is 15 to 40 times more prevalent than clinical form, it usually precedes the clinical form, it is difficult to detect and adversely affects milk quality and production, beside that it consider a reservoir of microorganisms that lead to infection of other animals within the herds. Losses due to mastitis may even be high in developed countries because mastitis prevention practices like post milking dipping of teat and dry period therapy are not so far being carried out. California Mastitis Test can be used as screening test to detect sub-clinically infected udders of female camels ***(Bekele and Molla, 2001)***, as the degree of gel formation is related with the number of cells in milk ***(Abdurahman, 2006).*** CMT scores may varied based on severity of inflammation. The percent of sublinical cases of mastitis in Daraw (48%) was higher than that in Shalateen (22%). This variation may be attributed to the difference of geographical area and individual herd management ***(Guidry, 1985).***

In present study the data of CMT showed that, the infection of udder in she camels was mainly subclinical and the prevalence of infection in Daraw and Shalateen was higher than that recorded by ***(Saber et al., 2010)*** who concluded that, the prevalence of subclinical mastitis was (9.52%), such variation may be attributed to the environmental factors and management that play a significant role in the

prevalence of subclinical mastitis ***(Abdurahman, 1996)***. Furthermore, in Daraw, camels were housed in closed places with other animals that may help in transmission of infection from other dairy animals. In addition to poor management lead to the high prevalence of the disease in the camel herds. In Shalateen the dry weather and rearing animals in open area may be the cause of decreasing the prevalence of infection in this area. However, the existence of the ticks and thorny plants may cause injury to the udder causing tissue damage that facilitate the entrance of microorganisms into udder ***(Woubit et al., 2001)***. These results revealed that, it is cheaper and easier to prevent mastitis by improving hygienic measures and culling chronically infected camels to eliminate important pathogen reservoirs, than to treat by medication and increase the cost of treatment including veterinary fees, medicines, risk of quackery and loss of milk production. In addition to that, the treatment also contribute to the buildup of antibiotic resistance.

The PCR method compared to conventional methods of identification of *S.aureus* isolates, the PCR method is less laborious and more accurate. In the future it is likely to be the predominant method of identification of pathogenic bacteria. In the present study, the identification of *S.aureus* isolates was performed conventionally and with the PCR method and detection of staphylococcal enterotoxins (sea, seb, sec) was performed using PCR method.

The current study indicated that, using culture method identified about 20% of examined samples infected with staphylococcal isolates including (9.3%) *S.aureus*. These results were close to some extent to that, recorded by ***(Abdel Hameid et al., 2004)***, who recorded that 14% of

their tested samples were positive to *S.aureus*, while the results of this study were lower than the results of ***(Türkyilmaz et al., 2010)*** and ***(El-jakee et al., 2008)*** who recorded 22.9% and 24.8%, respectively were positive to *S.aureus*.

Identification of these isolates was performed using biochemical method, 46.7% of these isolate showed positive for coagulase test. These results were higher than that previously reported by ***(Abdel All et al., 2010)*** as he recorded that 33.6% of isolates were positive for coagulase test. the present results were lower than ***(Mohamed, 2012)*** as he recorded 98.5% of isolates were positive for coagulase test.

All our isolates were not confirmed as *S.aureus* based on results from PCR, this may indicate that, PCR assay is more accurate test for detection of coagulation of *S.aureus* rather than, coagulase test, however, the later test is cheaper than the former technique. The production of coagulases is not unique feature of *S.aureus* but are shared by *S.intermedius* and *S.hyicus* ***(El-Jakee et al., 2008)***.

The results of PCR in this study revealed that, the positive samples for *Staphylococcus* spp were 13 (92.9%) out of 14 isolates. Subclinical staphylococcal mastitis obtained in this study as a result of PCR (8.66%), this result is coincides to ***(Sindhu et al., 2010)*** who stated that (6.58%) of milk samples contain *Staphylococcus* spp by PCR. *S.aureus* are 9 (64.3%) out of 14 isolates. subclinical mastitis caused by *S.aureus* (6%), this result similar to ***(Yesim and Haluk, 2012)*** was (6%) and close to results of ***(Fox and Gay, 1993)*** as they stated that *S.aureus* mastitis ranged from (7- 40%), while it was lower than that, recorded by ***(Janosi and Balty, 2004)*** who recorded 80%.

The presence of 3 enterotoxin genes (sea, seb and sec) was tested in 14 coagulase-positive Staphylococcus (CPS). 7 (50%) *Staphylococcus* isolates were enterotoxin-positive to sea, 5 (35.7%) of them for *S.aureus* strains and 2 (14.3%) for other coagulase positive spp. and these result close to ***(Da Cunha et al., 2007)*** as they stated that amongst the predominant Staphylococcus species, 100 % of the *S. simulans*, 64.7 % of *S. epidermidis*, 38.5 % of *S. saprophyticus* and 22.7 % of *S. aureus* harboured enterotoxin genes. *S. epidermidis*, *S. simulans*, *S. saprophyticus*, *S. hyicus* and *S. lentus* have been reported elsewhere to contain enterotoxigenic genes.

In the present study staphylococcal enterotoxin (SEA) 50% of subclinical mastitis. This result was similar to ***(Balaban and Rasooly, 2000)*** who recorded that the sea gene was the most prevalent gene. SEA is considered to be a primary cause of food poisoning ***(Cha et al., 2006)***.

This study concluded that raw milk may contain very dangerous pathogenic bacteria that make milk unsafe, capable of causing milk borne diseases.

The antimicrobial sensitivity test is important guide to the veterinarian in selecting the most appropriate antimicrobial agent for treatment of clinical mastitis and subclinical mastitis caused by *S.aureus*. The antimicrobial sensitivity testing of isolated strains (n=14) to various antimicrobials revealed that,(100%) of isolates were resistant to penicillin G and chloramphenicol followed by colistin (93%), tetracycline and lincomycin (78.6%), oxalinic acid (71.4%), amoxicillin (50%). The isolates showed that, the susceptibility to gentamycin (64.3%), neomycin and cefaclor (42.8%). The test show intermediate

susceptible result to ciprofloxacin (64.3%) and erythromycin (50%). The results of penicillin (100%) were close to result of ***(Abera et al., 2010)*** (94.4%), but higher than ***(Ebrahimi et al., 2009)*** (78.5%) and ***(Moroni et al., 2006)*** (69.1%). The result of erythromycin (35.7%) were closer to the result obtained from ***(Adwan, 2006*)** (40.9%), but higher than those reported by ***(Mohamed, 2012)*** (15.7%) and ***(Saad et al., 2007)*** (13.8%). The result of amoxicillin (50%) is higher than those reported by ***(Mohamed, 2012)*** (34.2%), but less than the results obtained from ***(Moroni et al., 2006)*** (100%) and ***(Klimien et al., 2011)*** (81.3%). In this study the antimicrobial susceptibility test of the staphylococcal isolates indicated that, most of isolates were susceptible to antimicrobial agents such as gentamycin, cephaclor followed by ciprofloxacin which are a new drugs. The susceptibility of isolates to antibacterial varies from strain to strain and from region to another, therefore the obtained data are in agreement with the results of Iraqi study ***(Al-Ani and Al-Shareefi, 1997)***. The ideal properties of drug selected for treatment of mastitis must has good tissue penetration, a low degree of protein binding, low irritancy and a short milk with holding time ***(Mody et al., 1998)***.

In conclusion *S.aureus* subclinical mastitis was common in milking she camels in Daraw and Shalateen affecting milk yield and consumer safety. California Mastitis Test, culture method and biochemical tests were not enough for detection of *S.aureus* subclinical mastitis. PCR assay is accurate, rapid and specific method for detection of *S.aureus* subclinical mastitis in milking she-camels. The caution and restricted prescription is recommended in order to avoid development of resistant bacterial strains and to avoid antibiotic residues in milk.

REFERENCES

- ***Abdel All, A. A. A.; Bashandy, M. M.; Yasin, M. H. and Ibrahim, A. K. (2010):*** Assessment of conventional and molecular features of *Staphylococcus aureus* isolated from bovine raw milk samples and contact dairy workers, Global Veterinaria 4(2): 168-175.
- ***Abdel Hameid, K. G.; Sender, G.; Prusak, B. and Ryniewicz, Z. (2004):*** Multiplex PCR protocol for diagnosis of cow udder infection with *Staphylococcus aureus*, Animal Science Papers and Reports vol.22 no.4:679-685.
- ***Abdurahman, O.A., (1996):*** The detection of subclinical mastitis in the Bactrian camel (Camelus bactrianus) by somatic cell count and California mastitis test. Vet. Res. Commun. 20: 9–14.
- ***Abdurahman O. A. Sh. (2006):*** udder health and milk quality among camels in the erred valley of eastern Ethiopia. division of comparative reproduction, obstitirics and udder health, live stock research for rural development. Vol.18(8)**.**
- ***Abera, M.; Abdi, O.; Abunna, F. and Megersa, B. (2010):*** Udder health problems and major bacterial causes of camel mastitis in Jijiga, Eastern Ethiopia: implication for impacting food security. Hawassa University, faculty of veterinary medicine, hawasse, Ethiopia. Trop. Anim. Health and Production., 42(3): 341-347.
- ***Adwan, G., M. (2006):*** Antibiotic resistance against staphylococcal isolates recovered from subclinical mastitis in the North of Palestine. Islamic University Journal, 14(1):1-9.

- ***Al-Ani, F.K. and AL-Shareefi, M.R (1997):*** Studies on mastitis in lactating one-humped camels (camelus dromedaries) in Iraq. J. Camel practical. Research. 4: 47-49.
- ***Al-Majali, A. M.; Zuhair Bani Ismail ; Yasseen AL-Hami and Abdelafatah Y. Nour, (2007):*** Lactoferrin concentration in milk from camels (camelus dromedarius) with and without subclinical mastitis. International journal research Vet. Med. Vol. 5 No.3 P. 120-124.
- ***Al-Otaibi, and El-Demerdash, (2013):*** Nutritive value and characterization properties of fermented camel milk fortified with some date palm products chemical, bacteriological and sensory properties. International Journal of Nutrition and Food Sciences, 2(4):174-180.
- ***Balaban, N., Rasooly, A., (2000):*** Staphylococcal enterotoxins. Int. J. Food Microbiol. 61: 1–10.
- ***Bekele, T., Molla, B., (2001):*** Mastitis in lactating camels (Camelus dromedarius) in Afar Region, north-eastern Ethiopia. Berl. Munch. Tierarztl. Wochenschr. 114: 169–172.
- ***Brakstad, O. ; Aasbakk, G.K. and Maeland, J. A.(1992):*** Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. Journal of Clinical Microbiology 30(7): 1654 -1660.
- ***Cha, J.O.; Lee, J. K.; Jung, Y. H.; Yoo, J. I.; Park, Y. K.; Kim, B. S. and Lee, Y. S.(2006):*** Molecular analysis of Staphylococcus aureus isolates associated with staphylococcal food poisoning in South Korea. J. Appl. Microbiol. 101: 864–871.

- ***Cookson, B. D. (1997):*** *Staphylococcus aureus*. In principles in clinical bacteriology, edited by M. Emmerson, C. Kibbler and P. Hawkey, John Willey Oxford 109-130.
- ***Cremonesi, P.; Luzzana, M.; Brasca, M.; Morandi, S.; Lodi, R.; Vimercati, C.; Agnellini, D.; Caramenti, G.; Moroni, P. and Castiglioni, B.(2005):*** Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. Mol. Cell Probes 19: 299–305.
- ***Da Cunha M. L.; Calsolari, R. A. and Junior, J. P.(2007):*** Detection of enterotoxin and toxic shock syndrome toxin 1 genes in Staphylococcus with emphasis on coagulase-negative staphylococci. Microbiol Immunol. 51: 381-390.
- ***Ebrahimi, A. and Akhavan Taheri, M. (2009):*** Characteristics of staphylococci isolated from clinical and subclinical mastitis cows in Shahrekord, Iran. Iranian J. vet. Res.,10: 270-277.
- ***El-Jakee, J. ; Nagwa, A. S.; Bakry, M.; Zouelfakar, S. A.; Elgabry, E. and Gad El-Sead, W. A. (2008):*** Characteristics of Staphylococcus aureus strains isolated from Human and Animal Sources, American-Eurasian J. Agri. & Environ. Sci.,4(2): 221-229.
- ***Fox, L. K. and Gay, J. M. (1993):*** Contagious mastitis. Vet. Clin. N. Am. Food Anim. Pract. 9(3): 475-488.
- ***Gilmour, A. and Harvey, J.,(1990):*** *Staphylococci* in milk and milk products. Soc. Appl. Bacteriol. Symp. Ser. 19:147–166.

- ***Guidry A. J. (1985):*** Mastitis and the immune system of the mammary gland. Larson, B. L. (Ed), Lactation. The Iowa State University Press, Iowa, P. 229-262.

- ***IDF (International Dairy Federation) Bovine mastitis (1987):*** Definitions and guide lines for mastitis diagnosis Bulletin – International Dairy Federation No. 211, Brussels, Belgium.

- ***Janosi, S. and Baltay, (2004):*** Correlation among the somatic cell count of individual bulk milk , result of California Mastitis Test and bacteriological status of the udder in dairy cows, Acta Vet Hung. 52: 173-183.

- ***Johnson, W.M.; Tyler, S.D.; Ewan E.P.; Ashton F.E.; Pollard D.R. and Rozee, K.R.(1991):*** Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in Staphylococcus aureus by the polymerase chain reaction. J. Clin. Microbiol. 29: 426–430.

- ***Klimien, M.; Špakauskas, V.; Matusevi Ius , A.; Mockeli Nas, R.; Pereckien, A.; Butrimait-Ambrozevi Ien and Virgailis, M. (2011):*** prevalence of gram positive bacteria in cow mastitis and their susceptibility of beta lactam antibiotics ISSN 1392-2130. Veterina riajia Irzootechnika (Vet Med Zoot). T. 56(78).

- ***Kuzma, K., Malinowski, E., Lassa, H., Klossowska, A., (2003):*** Detection of genes for enterotoxins and toxic shock syndrome toxin-1in *Staphylococcus aureus* isolated from bovine mastitis. Bulletin of the Veterinary Research Institute in Pulawy 47: 419–426.

- ***Lancett, G. A. and Bennett, R. W. (2001):*** *staphylococcus aureus* and staphylococcal enterotoxins. In : Downes , F. P. and Ito, K. (Eds). Compendium of methods for the micrbiological examination of foods, 4th edition. American Public Health Assiciation (APHA). Washington, D. C. USA.

- ***Le Loir, Y.; Baron, F. and Guatier, M. (2003):*** *Staphylococcus aureus* and food poisoning. Genet Mol Res. 2:63-76.

- ***Mody, S. K.; Patel, P. R. and Prajapati, C. B. (1998):*** A study on antimicrobial Susceptibility of bacteria isolated from the mastitic milk of rural camels in India. Proceeding of the third annual meeting for animal production under arid condition, vol. 2: 138-144, United Arab Emirates University.

- ***Mohamed, M, M. H. (2012):*** Penicillin resistance against staphylococcal isolates recovered from subclinical mastitis in Sohag Governorate, M. VSc. In Microbiology Dept. Vet. Med. South Valley University.

- ***Monday, S.R. and Bohach G.A.(1999):*** Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. J. Clin. Microbiol, 37: 3411-3414.

- ***Moroni, P.; Pisoni, G.; Antonini, M.; Villa, R.; Boettcher, P. and Carli, S. (2006):*** Antimicrobial drug susceptibility of Staphylococcus aureus from subclinical bovine mastitis in Italy .J. Dairy. Sci. 89(8): 2973-2976.

- ***National Mastitis Council (NMC), (1990):*** Procedures for collecting milk samples. In Microbiological Procedures for the Diagnosis of Bovine Under Infection (3rd Ed.).

- ***National Committee for Clinical Laboratory Standards "NCCLS" (2001):*** Performance standards for antimicrobial susceptibility testing . Supplement M100-S11. Villanova, PA, USA.
- ***Obied A.I ., Bagadi H.O. and Mukhtar M.M (1996):*** Mastitis in camelus dromedarius and the somatic cell content of camel's milk . faculty of veterinary science, university of Khartoum, Sudan, res. Vet. Sci. 61(1): 55-58.
- ***Reischl, U.; Pluz, M.; Ehret, W. and Wolf, H. (1994):*** PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. Bio. Techniques, 17: 844 - 845.
- ***Saad Gooraninejad; Masoud Ghorbanpoor and Amir Parviz Salati. (2007):*** Antibiotic susceptibility of Staphylococci isolated from bovine subclinical milk mastitis. Pakistan Journal of Biological Science 10(16): 2781-2783.
- ***Saber K., Sayed, M.. and Abdel-Rady, A. (2010):*** Sanitary condition of lactating dromedary she-camel environment with special references to milk quality and subclinical mastitis monitoring. Emirates Journal of food and Agriculture Vo1.22 No.3: 207-215.
- ***Sambrook, J. and Russel, D. (2001):*** Molecular Cloning: A Laboratory Manual, 3rd edition, Vol 1and 2, Cold Spring Harbor Laboratory Press, New York, USA.
- ***Schalm, O. W. and Noorlander, D. O. (1957):*** Experiments and observations leading to the development of California mastitis test. J. Am. Vet. Med. Asso., 130:199-204.

- ***Schrick, F.N.; Hockert, M.E.; Saxton, A.M.; Lewis, M.J.; Dowlen, H.H. and Oliver, S.P. (2001):*** Influence of subclinical mastitis during early lactation on reproductive parameters. J. Dairy Sci., 84: 1407-1412.
- ***Seifu, E. and Bekele, T.(2010):*** Prevalence and etiology of mastitis in traditionally managed camels (camelus dromedarius) in selected pastoral areas in eastern Ethiopia. Department of animal sciences, haramaya university, Dire dawa, Ethiopia veterinary journal. vol.14 no.2.
- ***Shearer, J. K. and B. Harris, J. R. (2003):*** Mastitis in dairy goat . Anim. Sci. Dept. Florida Coop. Ext. Serv. Inst. Food Agri. Sci. Univ. Fl., UAS. P. 1-6.
- ***Sindhu, N.; Sharma, A. and Jain, V.K. (2010):*** Diagnosis of Staphylococcal mastitis directly from milk of murrah buffaloes and cross bred cows by 16S-23S ribosomal RNA intergenic spacer PCR analysis. Israel Journal Of Veterinary Medicine. Volume 65(1): 23-26.
- ***Stephan, R., Annemüller, C., Hassan, A.A., Lämmler, Ch., (2001):*** Characterization of enterotoxigenic *Staphylococcus aureus* strains isolated from bovine mastitis in north - east Switzerland. Veterinary Microbiology 78: 373–382.
- ***Türkyilmaz, S.; Yildiz, ö.; Oryasin, E.; Kaynarea, S. and Bozdoğan, B.(2010):*** Molecular identification of bacteria isolated from dairy herds with mastitis, Kafkas Univ. Vet. Fak. Derg. 16(6):1025-1032.

- ***Vautor, E., Abadie, G., Guibert, J.M., Chevalier, N., Pe´ pin, M., (2005):*** Nasal carriage of *Staphylococcus aureus* in dairy sheep. Vet. Microbiol. 106: 235–239.

- ***Woubit,S,. Bayleyegen,M., Bonnet,P. and Jean-Baptiste, S. (2001):*** Camel (camelus dromedarius) mastitis in borena lowland pastoral area, southwestern Ethiopia. Revue Elev. Med. Vet. Pays trop., 54 (3-4): 207-212.

- ***Wielgosz-Groth, Z and Groth, I (2003):*** Effect of the udder health on the composition and quality of quarter milk from black-and white cows. Electron. J. Pol. Agr. U. Anim. husbandry, 6, Issue 2. http://www.ejpau.media.Pl/series/volume6/issue2/animal/art- 01.html.

- ***Yesim Cana, H. and Haluk Çelikb, T. (2012):*** Detection of enterotoxigenic and antimicrobial resistant *S. aureus* in Turkish cheeses. Food Control 24:100-103.

- ***Zinn C.E. (1999):*** Annual Report on *Staphylococcus aureus*. Denmark: *Staphylococcus* Laboratory, Staten's Serum Institute, 2000.