Bacteriological and Molecular Studies on some Bacteria Isolated From Mastitic Cattle and Humans Contact

[1]Ashraf A. Abd EL-Tawab, [1]Fatma, I. El-Hofy, [2]Khalid I. El-ekhnawey and[3]Fawzia A. El-Shenawey

[1]Bacteriology, Immunology and Mycology Department Faculty of Veterinary Medicine, Benha University
[2]Animal Health Research Institute.Dokki, Egypt
[3]Animal Health Research Institute, tanta, Egypt

A B S T R A C T

This study was done on a total of 92 mastitic milk samples (50 clinical, 42 sub-clinical) and 40 hand swabs from contact humans were collected from different dairy farms at Gharbia governorate. The collected samples were examined bacteriologically to isolate mastitis pathogens (*Staph. aureus*, *Strept. agalactiae*, *S. dysgalactiae* and *Strept. uberis*). From clinical mastitic samples, six isolates were *S. aureus* (12%) and one isolate was (2%) *S. dysgalactiae*. Among sub clinical mastitic milk samples two isolates were *S. aureus* (4.6%) and one isolate (2.3%) *S. agalactiae*. While *S. uberis* were not detected. From contact human hand swabs both *S. aureus* and *Streptococcus* species were not detected. Antibiotic sensitivity test revealed that all bacterial isolates were highly sensitive to enerofloxacin, ciprofloxacin, sulpha |trimethoprim and gentamicin respectively, while all isolates were resistant to penicillin followed by amoxicillin/Clavulanic acid. Two isolates of *S. aureus* were screened for detection of enterotoxin genes (Sea, *Se*b, *Se*c, *Se*d and *Se*e) by multiplex PCR. Only *Se*d gene was detected in one isolate. *Cfb* gene (CAMP factor) and *hyl* (hyluronidase) gene were detected in *S. agalactiae*. *mig* (surface-expressed *mig* protein) gene was detected in *S. dysgalactiae*.

Key words: Mastitic bacteria, Cattle, Humans contact

(http://www.bvmj.bu.edu.eg) (bvmj, 34(1): 66 -78, MARCH, 201

1. INTRODUCTION

Mastitis is an important disease that limits dairy production. The disease should be studied as it causes financial loss as a result of reduced milk yield, discarded milk following antibiotic therapy, veterinary expense and culling of mastitic cows (Radostitis *et al.*, 2007). It is primarily resulting from an invasion of mammary tissues by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological changes in glandular tissues and milk (Quinn *et al.*, 2002 and Radostitis, 2007) Many infectious agents are responsible in causing the disease in dairy animals as bacterial agents like *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Corynebacterium spp.*, *Klebsiella spp.*, *Psudomonas spp*., Mycoplasmal agents, fungal agents, viral agents are responsible for the disease (Radostits et al., 1995). Mastitis can occur in a clinical and sub clinical form, the latter is commonly occurring in most

herds (Gruet et al., 2001 and Awale et al., 2012). The controls of mastitis in dairy herds are accomplished in part with the aid of antibiotics therapy (NMC, 1999).

Staphylococcus aureus is a versatile pathogen responsible for a variety of infections in humans and animals (Hata et al., 2008). The production of enterotoxins is particularly significant from a public health standpoint as the ingestion of preformed toxins is a major cause of food poisoning worldwide (Le Loir et al., 2003 and Srinivasan et al., 2006).

S. agalactiae is a highly contagious agent and commonly found in the mammary gland of cattle (Fonseca and Santos.,2000) and usually associated with acute clinical -mastitis and persistent subclinical infections (Hillerton et al., 2004). The molecular tests on *S. agalactiae* indicated the presence of virulence genes as *fbs*A (encoding fibrinogen-binding protein),and *hyl*(encodes hyalurinidase enzyme) which play a role in pathogenesis of *S. agalactiae* (Arbini et al., 2016).

The *fbs*A gene is responsible for encoding the protein *fbs*A , which allows the binding of *S. agalactiae* to fibrinogen, soluble or mobilized from extracellular matrix of the host organism (Sukhnanand et al., 2005). The adherence of *S. agalactiae* to host tissues is important in the early infection process (Frost et al.,1977 and Rosini et al 2006), and recent studies have shown that the protein *fbs*A also has platelet function and may cause other problems during infection (Pietrocola et al.,2005), but may also be involved escape mechanism in the immune system, preventing opsonization by macrophages and neutrophils (Sukhnanand et al., 2005).

The gene is responsible for *hly*B protein called hyaluronatelyase [*hly*B], which is very important for the pathogenesis of *S. agalactiae* (Glazer et al.,2002). This protein belongs to a special group of enzymes called hyaluronidase that responsible for the degradation of polysaccharides such as chondroitin, chondroitin sulfate, and especially the N acetyl glucosamine, which is part of the composition of hyaluronic acid facilitating the spread of *S. agalactiae* during infection (Akhtar et al., 2006).

Among the environmental streptococci, *S. dysgalactiae* is one of the most prevalent, which may infect mammary glands as favorable conditions are present (Todhunter et al., 1995). It can produce a surface-expressed M- like proteins called *mig*, which promote dissemination of the organism into host tissue (calvinho et al., 1998).

So, the current study aimed to isolate and identify some bacteria from mastitic milk and human contact and to detect some of their virulence genes using biochemical tests and PCR respectively.

2. MATERIAL AND METHODS:

2.1. Sampling : a total of 92 mastitic milk samples (50 clinical, 42 sub-clinical) and 40 hand swabs from contact humans were collected from different dairy farms at Gharbia governorate.

A. Milk samples:

Mastitis milk samples were collected aseptically into screw capped bottles and kept at 4^{o}C until microbiological examination.

Twenty five ml from each sample were homogenized with 225 ml of buffered peptone water (BPW) for pre-enrichment and incubated at 37^{o}C for 24 h (Addis *et al.*, 2011).

B. contact human hand swabs:

Moistened sterile swabs were rolled over the palm of hands, finger tips , nails and area between fingers of human contacts. Each swab was inserted in tubes containing BPW

for pre- enrichment.

2.2. Bacterial isolation by cultivation: A loopful from the pre-enriched culture homogenate in BPW was streaked onto the surface of Baird Parker agar , mannitol salt agar and Edward's medium. The inoculated plates were incubated at 37°C for 24 to 48 hours then examined for bacteriological growth. Suspected colonies which appeared on different media were sub cultured, purified, and preserved in semisolid agar for further identification. Bacterial colonies were identified morphologically and microscopically using Gram stain as well as biochemically using methods described by Koneman et al., (1988) and Quinn et al., (2002).

2.3. Antimicrobial susceptibility testing : It was done according to Quinn et al. (1994) and Winn et al. (2006): The obtained bacterial isolates were tested in vitro for their susceptibility to the following antimicrobial discs: enerofloxacin (Enr 10), ciprofloxacin (Cip5), penicillin (P10), amoxicillin/ Clavulanic acid (Amc 10), oxytetracyclin (OT 30), gentamicin (Gen 30) and sulpha trimethoprim (Sxt25).

2.4.Extraction of bacterial DNA :

DNA was purified according to QIAamp DNA mini kit instructions.

2.5. Multiplex PCR for identification of Streptococcus species:

Purified DNA of *S. agalactiae, S. dysgalactiae* and *S. uberis* isolates was subjected to a multiplex PCR for the identification according to (Raemy et al., 2013) as shown in the table(1).

2.6.Multiplex PCR for detection of Staph. aureus enterotoxins :

Purified DNA of *Staph. aureus* isolates was subjected to a multiplex PCR for the identification of enterotoxins according to (Mehrotra et al., 2000).as shown in the table (2) and agarose gel electrophoreses according to (Sambrook et al., 1989) with agarose gel (1.5 g).

Cycling conditions of the primers during cPCR : Temperature and time conditions of the two primers during PCR are shown in table (3) according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit.

3. RESULTS:

Incidence of bacterial species: from clinical mastitic milk samples a total of six isolates of *S. aureus* (12%) and one isolate (2%) of*S. dysgalactiae* were isolated. Among sub clinical mastitic milk samples two isolates of *S. aureus*(4.6%) and one isolate (2.3%) of *S. agalactiae*. while *S. uberis* were not detected. In contact human hand swabs no *S. aureus* and no *Streptococcus* species were detected.

Antibiotic sensitivity test results:

Antibiotic sensitivity determination revealed that all bacterial isolates were susceptible to enerofloxacin (100%), ciprofloxacin (90%), sulpha /trimethoprim and gentamicin (70%). Moderate sensitivity to oxytetracyclin (40%). On the other hand all isolates were resistant to penicillin followed by amoxicillin/Clavulanic acid.

Detection of enterotoxin genes in *Staph. aureus* by multiplex PCR: Two isolates of *S. aureus* were screened randomly for detection of *enterotoxin* virulence genes by multiplex PCR and the result revealed that one isolate contain only *Sed* gene.

Detection of virulence genes in *S. agalactiae* and *S. dysgalactiae* by PCR: *cfb*gene (CAMP factor) and *hyl*(hyluronidase) gene were detected in *S. agalactiae*. *mig* (surface-expressed *mig* protein) gene was detected in *S. dysgalactiae* .

Table (1): Designing of primers used for *Streptococcus* species identification:

Target gene	sequence Primer		Amplified product	Reference
S. agalactiae cfb (CAMP factor)	*F*	TTTCACCAGCTGTATTAGAAGTA	153 bp	Ke *et al*., 2000
	R	GTTCCCTGAACATTATCTTTGAT		
S. agalactiae hyl	F	CATACCTTAACAAAGATATATAA CAA	950 bp	Krishnaveni *et al*., 2014
	R	AGATTTTTTAGAGAATGAGAAGTTTTTT		
S. dysgalactiae mig	F	CGTTTTTAGTTTCGGGAGCA	188 bp	
	R	TGCCTTCAATTGAGTCTGCTG		

Table (2) : Designing of primers used for detection *Staph. aureus* enterotoxins:

Target gene		Sequence	Amplified product	Reference
Sea	*F*	GGTTATCAATGTGCGGGTGG	102 bp	Mehrotra *et al*., 2000
	R	CGGCACTTTTTTCTCTTCGG		
Seb	*F*	GTATGGTGGTGTAACTGAGC	164 bp	
	R	CCAAATAGTGACGAGTTAGG		
Sec	*F*	AGATGAAGTAGTTGATGTGTATGG	451 bp	
	R	CACACTTTTAGAATCAACCG		
Sed	*F*	CCAATAATAGGAGAAAATAAAAG	278 bp	
	R	ATTGGTATTTTTTTTCGTTC		
See	*F*	AGGTTTTTTCACAGGTCATCC	209 bp	
	R	CTTTTTTTTCTTCGGTCAATC		

Table (3): Cycling conditions of the primers during cPCR :

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. aureus enterotoxin	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
Streptococcus virulence genes	95˚C 15 min	*94˚C 60 sec*	54.6 C 60 sec.	72˚C 60 sec	35	72˚C 10 min.

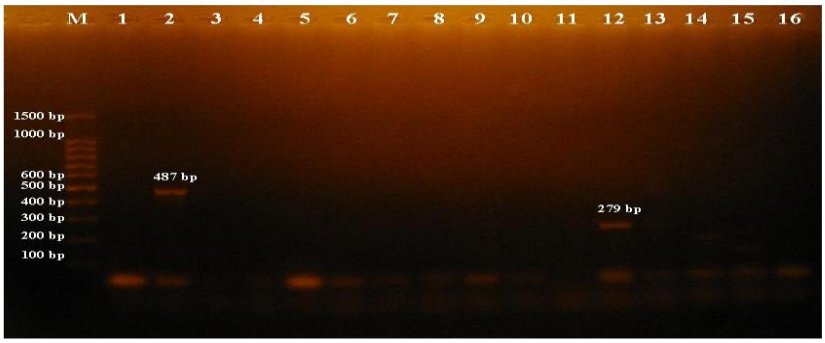

Figure (1): Gel electrophoresis pattern of multiplex PCR for *Streptococcus* species identification: Lane (2): amplification of *skI*A3 gen of *S. agalactiae* at 487 bp . Lane (12) : amplification of *16S* RNA gene of *S. dysgalactiae* at 279 bp.

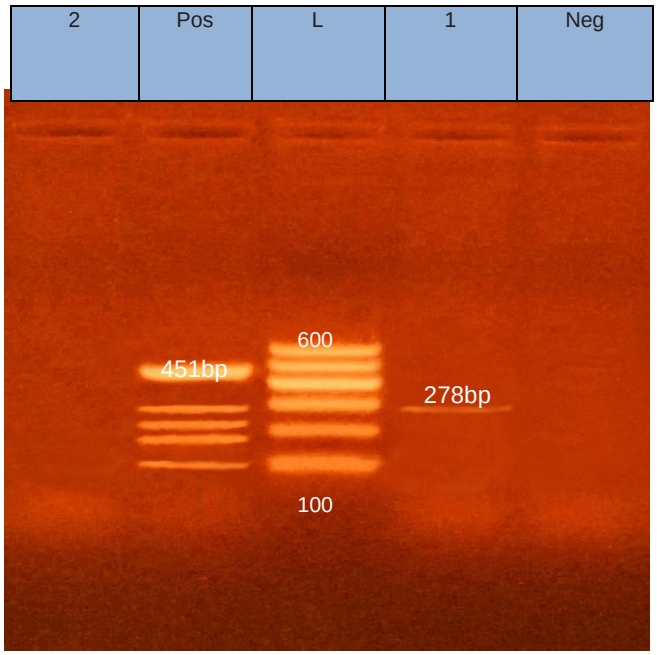

Figure (2): Gel electrophoresis pattern of multiplex PCR for detection of enterotoxingenes of *S. aureus* : Lane (1) positive amplification of *Se*dgene at 278 bp, L: ladder from 100 bp to 600 bp, Pos: positive control, N: Negative control.

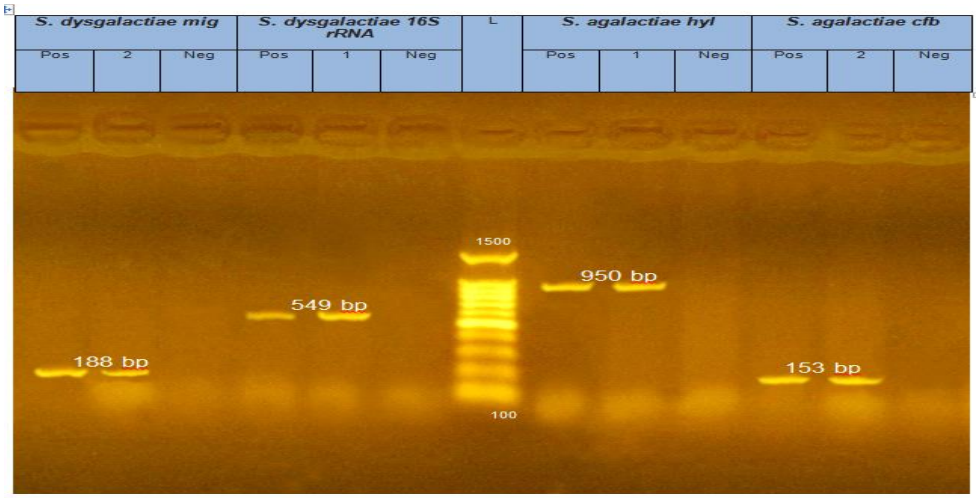

Figure (3): Gel electrophoresis pattern for detection of some virulence genes of *S. agalactiae* and *S. dysgalactiae* : At right lane 1 positive amplification of *hyl* gene at 950 bp , lane 2 positive amplification of *cfb* gene at 153bp . At left lane 1 positive amplification of *16S*rRNA gene, Lane 2 positive amplification of *mig*gene at 188 bp.

4. DISCUSSION:

Regarding to obtained data *Staph. aureus* was the major pathogen causing clinical mastitis with incidence of (12%). Similar result of (13.5%) from clinical mastitis was detected previously by Deif , (2011). Higher incidence of *Staph. aureus* (28.32%) was detected by Ali et al. (2011), (53.4%) by Alekish et al. (2013), and (43.3%) by Duguma et al. (2014). Lower *Staph. Aureus* isolation rate (6.7%) from clinical mastitis than the current study was previously reported by Sargeant et al. (1998), and (10%) by El-Dahshan and Nada (2015).

In the current study *S. aureus* was isolated with percentage of (4.6%) from sub-clinical mastitis. Higher incidence of *Staph. aureus* isolates from sub- clinical mastitis with percentage of (29%) was recorded by Calderon and Virginia (2008) and (52.2%) by Abdel-Rady and Sayed (2009), 35.71% by Abera et al. (2013) and (26.08%) by Hande et al. (2015). Higher incidence 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 infected gland also *S. aureus* is a contagious organism with capacity to penetrate into the tissue producing deep seated foci (Ranjan et al., 2011).

Staphylococcus aureus is the most important and prevalent contagious mammary pathogen. It causes clinical and subclinical intra mammary infection with serious economic loss and herd management problems in dairy cows (Dego et al., 2002). Nearly 95% of staphylococcal food poisoning are caused by SE types from SEA to SEE by (Tamarapu et al. 2001), SEA is considered the most commonly involved enterotoxin (Balaban and Rasooly, 2000). Such toxins are the more resistant and the ingestion of at least 1 gram of enterotoxin per 100 grams of food is enough to induce food poisoning (Tranter, 1996) ; Cremonsei et al., 2005). Therefore, the determination of SEs producing strains in food is important with respect to assessing public health risks (Aydin et al., 2011).

In the present study two isolates of *S. aureus* were examined randomly for detection of enterotoxin virulence genes (*Se*a, *Se*b, *Se*c, *Se*d and *Se*e) by multiplex PCR, only *Se*d gene was detected . this result agreed with the results obtained by Elsayed et al. (2015) who detected enterotoxine type D on *Staphylococcus aureus* isolated from clinical and subclinical mastitis. While Abeer et al.(2010) detected *Se*a and *Se*b genes in bovine milk samples. On the other hand Mohamed et al. (2016) reported that none of *Staph. aureus* strains which isolated from mastitic milk samples produce enterotoxins A, B, C, D or E.

Milkers' hands are considered as an initial point of contamination with *Staph. aureus* in dairy farms (Olivindo et al., 2009). Nearly, 30-50% of humans carry *Staph. aureus* and one third to one half of the organisms have been shown to be

enterotoxigenic (Bergdoll, 1989).

Regarding to isolation of *Staph. aureus* from contact human hand swabs in the present study, it was not isolated from contact human hand swabs . In other studies, the isolation rate of *Staph. aureus* from milkers' hand swabs were previously reported by Lee et al. (2012) with percentage of 3.3% in Brazil and 45.9% by Adesiyun et al. (1998) in Trinidad. Moreover, an isolation rate of 44.1% was reported in skin swabs of dairy workers in Aswan, Egypt by Abdel-All et al. (2010) and was isolated with percantage of 10% from milkers'hand swabs by El-Gedawy et al. (2014) in Sharkia, Egypt.

Streptococcus species as S. *agalactie, S. dysgalactiae* and *S. uberis* have been reported as common causative agents for mastitis (Khan et al., 2003). PCR amplification of species-specific parts of the gene encoding the 16S rRNA and cfb gene, had been successfully used for the rapid and reliable identification of these species (Jayarao et al., 1992) and Picard et al. 2004).

In the present study it is clear that isolation of *Streptococci* has been limited by both species of *S. agalactiae* with percentage of (2.3%) from sub clinical mastitis and *S. dysgalactiae* with percentage of (2%) from clinical mastitis, while were not isolated from contact human hand swabs . These results were nearly similar to results of Heba (2011) who has isolated *S. agalactiae* with percent (3.1%) from clinical mastitic cases and (2%) from sub clinical mastitic cases and *S. dysgalactiae* with (1.2%) from clinical mastitic cases and (1.3%) from sub clinical mastitic cases. Also lower incidence of *S. agalactiae* were recovered by El-Zubeir et al. (2006), at rate of 0.83%. Lower isolation rate of *S. dysgalactiae* with (2.5%) and (4%) was recorded by Balakrishnan et al. (2004) and Turutoglu et al. (1995) respectively. On the other hand, higher incidences of *S. agalactiae* isolated from mastitic cows were recovered by Borkowoska et al. (2006) and Bi et al. (2016) with isolation rates of 84.8% and 92.2%, respectively. Higher isolation rate of *S. dysgalactiae* with 14% , 17% and 72.3% was recorded by Moges et al. (2011) , El Jakee et al. (2013) and Bi et al. (2016) respectively.

*S. agalactia*e is an obligate organism of the epithelium and tissue of the mammary gland. It can be eradicated from dairy herds, through detection and segregation of infected cows, using hygienic milking and intra mammary infusion of antimicrobial agents (Schalm et al., 1971); Gyles and Thoen (1993). Some virulence factors, including fibrinogen binding protein (*fnb*), hyaluronatelyase, and CAMP factor (*cfb*) are responsible for *S. agalactie* infections (Franken et al., 2001) and Beckmann et al., 2002). In the present study, PCR detection of cfb gene encoding for CAMP factor and *hyl* gene encoding for hyalurinidase gene of *S. agalactiae* revealed that, *cfb* gene was amplified at 153 bp. These results are in accordance with the results of Krishnaveni et al. (2014) and El-Gedawy et al. (2014) who

have been reported that *cfb* gene was detected in all *S. agalactiae* isolates. *hyl* gene was detected and amplified at 950 bp. Other study by Ayman et al. (2015) reported that hyl gene was detected in *S. agalactiae* isolated from milk samples with (81.39%), while Clarisse (2011) and Abdel-Tawab et al. (2017) reported that (38.8%) and (25%) respectively of *S. agalactiae* isolates contain *hyl* gene. Also *S. dysgalactiae* able to produce M-like protein called *mig* which involved in resisting phagocytosis by bovine neutrophils and spreed of infection Song et al.,(2001). In the current study *mig* gene of *S. dysgalactiae* was detected and amplified at 188 bp in the examined isolate. Our finding agreed with the finding of Abdel-Tawab et al. (2017) who recorded that *mig* gene of *S. dysgalactiae* was detected in the all examined isolates of *S. dysgalactiae*. However, Ibrahim et al. (2016) stated that *mig* gene was detected with percentage of (77.8%) of the examined isolates.

Antibiotic sensitivity determination revealed that all bacterial isolates (*S. aureus*, and *Streptococcus* spp) were fully susceptible to enerofloxacin (100%), followed by ciprofloxacin (90%) , sulpha /trimethoprim and gentamicin (70%). While moderate sensitivity to oxytetracyclin (40%). On the other hand all isolates were fully resistant to penicillin and amoxicillin/Clavulanic acid. These results go in parallel with the results of Al-ekish et al. (2013), Chandrasekaran et al., (2014), Idriss et al. (2014) , Yasin et al. (2016) and Tavakoli and Pourtaghi (2017).

5. CONCLUSION:

Detection of some bacteria as *S. aureus* and *Streptococcus* species from mastitic milk of cattle and contact human was found to be an important point for animal health and contact humans. Multiplex PCR can be used as rapid and accurate method for detection of *Streptococcus* mastitis and virulence genes of *Streptococcus* and *Staphylococcus* aureus. Enerofloxacin and ciprofloxacin were the most effective antibiotics on treatment of cattle mastitis.

6. REFERENCES:

Abdel-All, A. A. A.; Yasin, B. M. H. and Ibrahim, A. K. (2010): Assessment of conventional and molecular features of *Staphylococcus aureus* isolated from bovine milk samples and contact dairy workers. Global Veterinaria 4(2): 168-175.

Abdel-Rady, A. and Sayed, M. (2009): Epidemiological Studies on Subclinical Mastitis in Dairy cows in Assiut Governorate. Vet. World, 2(10): 373-380.

Abdel-Tawab, A.A.; Abou El-Roos, N.A.; El-Hofy, F.I. and Abdullah, H.E. (2017): Molecular studies regarding to virulence factors of Streptococcus species isolated from raw milk. Benha Veterinary Medical Journal, 32(1): 145-152.

Abeer, A.A.; Bashsndy, M.M.; Yasin, M.H. and Ibrahim, A.K. (2010):Assessment of conventional and molecular features of Staphylococcus aureus isolated from bovine milk samples and contact dairy workers. Global Veterinaria 4(2):168-175.

Abera, B.; Lemma, D. and Iticha, L. (2013) : Study of bovine mastitis in asella government dairy farm of Oromia Regional state, South Eastern Ethiopia : Int.J. Curr. Res. Aca. Rev.1(2) 134-145.

Addis, M.; Pal, M. and Kyule, M. N. (2011a): Isolation and identification of Staphylococcus species from raw bovine milk in DebreZeit, Ethiopia. Veterinary Research 4 (2): 45-49.

Adesiyun, A. A.; Webb, L. A. and Romain, H. T. (1998): Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. Journal of Food Protection 61 (5): 629-632.

Akhtar, M.d.; Krishnan, M.Y.; Bhakuni ,V. (2006)// : Insights into the mechanism of action of hyaluronatelyase: role of C-terminal domain and Ca2+ in the functional regulation of enzyme. J BiolChem 281: 2833-2834.

Al-ekish, M.O.; Al-Qudah, K.M. and Al-Saleh, A. (2013) : Prevalence of antimicrobial resistance among bacterial pathogens isolated from bovine mastitis in northern Jordan. *Revue Méd.Vét.*, 164 (6) : 319-326.

Ali, M.A; Ahmad, M.B.; Muhammad, K. and Anjum. (2011):Prevalence of clinical mastitis in dairy buffaloes of Punjap, Pakistan. Jour. of Anim. And Plant. Sciences.21(3):477-480. -Arbini, Cl.M.;Cardoso,P.G.; Paiva, I.M.; Custodio, D.A.C. and Costa, M.G.(2016): Virulence genes of the Streptococcus agalactiae associated with bovine mastitis in Minas Gerais Livestok Herds, Brazil. Applied Microbiology.2(3):1-7.

Awale, M.M.; Dudhatra,G.B.; Avinash, K.; Chauhan, B.N.; Kamani, D.R.; Modi, C.M.; Patel, H.B. and O·Kennedy, R. (2012): Bovine mastitis: at hreat to economy. Open Access Scientific Reports. 1, 295.Doi: 10.4172/scientific reports.295.

Aydin, A.; Sudagidan, M. and Muratoglu, K. (2011): Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *Staphylococcus aureus*strains isolated in the Marmara Region of Turkey. International Journal of Food Microbiology 148 (2): 99-106.

Ayman, El-B.; Mohamed, E.; Eman, M. and Yaser, B. (2015): Detection of virulence genes of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitis in Middle East. British Microbiology Researsh Journal. 10 (3):1-9.

Balaban, N. and Rasooly, A. (2000): Staphylococcal enterotoxins. International Journal of Food Microbiology 61 (1): 1-10.

Balakrishnan, G.; Unny, M.; Dorairanjan, D. and Subramanian, M.; (2004): Studies on bovine mastitis at Namakkal. Indian Veterinary Journal, 81(10): 1166-1167.

Beckmann, C.; Waggoner, J.D.; Harris, T.O.; Tamura, G.S. and Rubens, C.E. (2002):Identification of novel adhesins from Group B streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding. Infect Immun.; 70: 2869-876.

Bi, Y.; Wang, Y.J.; Qin, Y.; Vallverdú, R. G.; García, J.M.; Sun, W.; Li, S. and Cao, Z. (2016): Prevalence of Bovine Mastitis Pathogens in Bulk Tank Milk in China. PLoS ONE, 11(5).

Borkowska, D.; Polski and Janus, E. (2006): Microorganisms isolated from cow quarter milk and their susceptibility to antibiotics. Annales UniversitatisMariae Curie SkodowskaSectio EE Zootechnica., 24:27-32.

Calderon, A. and Virginia Rodriguez. (2008): Prevalence of bovine mastitis and its infectious etiology in specialized milk production systems at cundiboyacense plane (Colombia). Rev Colom CiencPecua., 21(4).

Calvinho, L.F.; Almeida, R.A. and Oliver, S.P. (1998): Potential virulence factors of *Streptococcus dysgalactiae* associated with bovine mastitis. Vet Microbiol., 61(1-2):93-110.

Chandrasekaran,D.; Rankatesan, P.; Tirumurugaan,K.G.; Nambi, A.P.; Thirunavukkarasu, P.S.; Kumanan, K.; Vairamuthu,S.; and Ramesh, S. (2014): Pattern

of antibiotic resistant mastitis in dairy cows. Veterinary World 7(6): 389-394.

Clarisse, M.A. (2011): Virulence genes of the Streptococcus agalactiae associated with bovine mastitis in livestock herds in Minas Gerais, Brazil. M.V.SC. Thesis, Federal University of Lavras.

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. Molecular and Cellular Probes 19 (5): 299-305.

Dego, O.K.; Dijk, J. E. V. and Nederbragt, H. (2002): Factors involved in the early pathogenesis of bovine Staphylococcus *aureus* mastitis with emphasis on bacterial adhesion and invasion: a review. Veterinary Quarterly, 24 (4): 181-198.

Deif, H.N.M. (2011): Polymerase Chain Reaction as an analytical tool in the diagnosis of Cattle mastitis. M.V.Sc. thesis (Microbiology), Fac. Vet. Med. Cairo Univ.

Duguma, A.; Tolosa,T. and Yohannes, A. (2014): Prevalence of clinical and subclinical mastitis on cross bred dairy cows at Holleta agricultural research center, Centeral Ethiopia ,Acadimic Journal of Vet .Med.and Animal Health, 6(1):13-17.

El-Dahshan, E.M. and Nada, S.M. (2015): Differentiation between *S. agalactia, S. dysagalactia and S. aureus* isolated from milk of mastitic cows by polymerase chain reaction. Benha veterinary medical journal, 28(1) :27-32.

El-Gedawy, A.A.; Ahmed, H. A. and Awadallah, M. A. I. (2014): Occurrence and molecular characterization of some zoonotic bacteria in bovine milk, milking equipments and humans in dairy farms, Sharkia, Egypt. International Food Research Journal; 21(5): 1813-1823.

El-Jakee, J.; Hableel, H.S.; Kandil, M.; Hassan, O.F.; A. Khairy, E. and Marouf, S.A. (2013): Antibiotic Resistance Patterns of Streptococcus agalactiae Isolated from Mastitic Cows and Ewes in Egypt. Global Veterinaria,10 (3): 264-270.

El-sayed, M.S, El-Bagoury, A.M, and Mai, A.D. (2015): Phenotypic and genotypic detection of virulence factors of Staphylococcus aureus isolated from clinical and subclinical mastitis in cattle and water buffaloes from different farms of Sadat City in Egypt . Vet World. 8(9): 1051-1058.

EZubeir, 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 (2): 101-109.

Fonseca, L.F.L .and Santos, M.V., (2000) : Qualidade do leite e controle de mastite. Lemos 1: 2.

Frost, A.J.; Wanasinghe, D.D. and Woolcock, J.B. (1977): Some factors affecting selective adherence of microorganisms in the bovine mammary gland. Infect Immun 15: 245-253.

Franken, C.; Haase, G.; Brandt, C.; Weber-Heynemann, J.; Martin, S.; Lämmler ,C. et al. (2001):Horizontal gene transfer and host specificity of beta-haemolytic streptococci: the role of a putative composite transposon containing scpB and lmb. MolMicrobiol. 41(4): 925-35.

Glazer, P.; Rusniok, C.; Buchrieser, C.; Chevalier, F.; Frangeul ,L. (2002):.- Genome sequence of Streptococcus agalactiae, a pathogen causing invasive neonatal disease. MolMicrobiol 45: 1499-1513.

Gruet, P.; Maincent, P.; Berthelot, X. and Kaltsatos,V., (2001): Bovine mastitis and intra mammary drug delivery: review and perspectives Adv. Drug. Deliv.Rev; 50,245-249.

Gyles, C.L. and Thoen, C.O. (1993): E. coli In: pathogenesis of Bacterial Infection in Animal. 2nd Edn. International Book Distributing Co., Lukhnow.164-187.

Hande, G.; Arzu, F.; Nilgün, G.; Serhat, A.S.; Alper, C.; Ece, K.; Serhat, A.and Murat, F. (2015): Investigation on the etiology of subclinical mastitis in Jersey and Hybrid jersey dairy. ActaVeterinaria-Beograd., 65 (3): 358-370.

Hata, E.; Katsuda, K.; Kobayashi, H.; Nishimori, K.; Uchida, I.; Higashide, M. et al. . (2008): Bacteriological characteristics of *Staphylococcus aureus* isolates from humans and bulk milk. J. Dairy Sci; 91, 564–569.

Heba, D.M.N. (2011): Polymerase Chain Reaction as an analytical tool in the diagnosis of Cattle Mastitis. Master Thesis. Faculty of Vet.Med-Cairo University.

Hillerton,J.E.; Leigh,J.A.; Ward, P.N. and Coffey, T.J. (2004):1 Streptococcus agalactiae infection in dairy cows. Vet Rec ; 154: 671-67.2.

Idress, SH.E.;Foltys, V.; Tancin, V.; Kirchnerova, K.; Tancinova, D. and Zaujec, K. (2014): Mastitis causing pathogens and their resistance against antimicrobial agents in dairy cows in Nitra, Slovakia, Slovak.J.Anim.Sci., 47(1):33-38.

Ibrahim, E.E.; Mena Alla, A.R.; Hisham, S.N. and Gamal, R.H.(2016): Molecular detection of *Streptococcus* species isolated from cows with mastitis. World's Veterinary Journal. 6(4):193-202.

Jayarao, B. and Dore, J., Oliver S.(1992): Restriction Fragment Length polymorphism Analysis of 16S Ribosomal DNA of *Streptococcus* and *Enterococcus* Species of Bovine Origin. J Clin Microbiol. 30: 2235-2240.

Ke, D.; Ménard, C.; Picard, F.J.; Boissinot, M.; Ouellette, M.; Roy, P.H. and Bergeron, M.G. (2000): Development of Conventional and Real-Time PCR Assays for the Rapid Detection of Group B Streptococci. *Clinical Chemistry* 46:3, 324–331.

Khan, I.U.; Hassan, A.A.; Abdulmawjood, A.; Lammler, C.; Wolter,W. and Zschock, M. (2003): Identification and epidemiological characterization of *Streptococcus uberis* isolated from bovine mastitis using conventional and molecular methods. Journal of Veterinary Science; 4(3):213-223.

Koneman, E.W.; Stephen, D.A.; William, M.J.; Herbert, M.S. and Washington, C.W. (1988): Diagno-stic Microbiology. 3rd ed.J.BLippin-cott CO.

Krishnaveni, N.; Isloor, S.K.; Hegde, R.; Suryanarayanan, V.V.S.; Rathnma, D.; Veeregowda, B.M.; Nagaraja, C.S. and Sundareshan, S. (2014):Rapid detection of virulence associated genes in *Streptococcal* isolates from bovine mastitis. African Journal of Microbiology Research ;8(22), pp. 2245-2254.

Lee, S. H. I.; Camargo, C. H.; Gonçalves, J. L.; Cruz, A. G.; Sartori, B. T.; Machado, M. B. and Oliveira, C. a. F. (2012): Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. Journal of Dairy Science 95 (12): 7377-7383.

LeLoir Y.; Baron F. and Gautier M. (2003): Staphylococcus aureus and food poisoning. Gen. Mol. Res.2, 63–76.

Mehrotra, M.; WANG, G. and, Johnson, W.M. (2000): Multiplex PCR for Detection of Genes for Staphylococcus aureus Shock Syndrome Toxin 1, and Methicillin Resistance. Journal of Microbiology ; 38, No. 3.

Moges, N.; Asfaw, Y. and Belihu, k. (2011): A Cross Sectional Study on the Prevalence of Sub Clinical Mastitis and Associated Risk Factors in and Aronund Gondar, Northern Ethiopia. International Journal of Animal and Veterinary Advances; 3 (6): 455-459.

Mohamed, E. A. N. and Rehab, E. D. (2016): A study on Bacterial and Fungal Causes of Subclinical Mastitis in Dairy Cows. Egypt. J. Chem. Environ. Health; 2 (2):425 -438.

NMC, (1999): Current Concept in bovine mastitis National mastitis Council (NMC). 3rd .1840 Wilson blud, Arlinton, VA 22201.

Olivindo, C. D. S.; Chapaval, L.; Villarroel, A. B. S.; Alves, F. S. F.; Sousa, F. G. C. D. and Fernandes, F. E. P. (2009): Detecção de *Staphylococcus aureus* utilizando a técnica de REP-PCR no monitoramento da qualidade do leite de cabra. Revista Brasileira de Zootecnia 38: 1317-1321.

Picard, F.; Ke, D.; Boudreau, D.; Boissinot, M.; Huletsky, A.; Richard, D.; Ouellette, M.; Roy, P. and Bergeron, M. (2004):Use of *tuf* Sequences for Genus-Specific PCR Detection and Phylogenetic Analysis of 28 Streptococcal Species. J Clin Microbiol. 42(8):3686–3695.

Pietrocola, G.; Schubert, A.; Visai, L.; Torti, M.; Fitzgerald, J.R. et al. (2005):// FbsA, a fibrinogen-binding protein from Streptococcus agalactiae, mediates platelet aggregation. Blood 105: 1052-1059.

Quinn,p.j.; Carter,M.E.; Markey, B.K. and Carter, G.R.(1994): Clinical veterinary microbiology, Mosby year book Europe limited, Lynton House, London; P. 109-126.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (2002): Clinical Veterinary microbiology. Harcourt publishers,Virginia, pp; 331-344.

Radostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2007): Mastitis In: Veterinary medicine 9th ed., Harcourt Ltd, London; 174-758.

Radostits, O.M.; Blood, D.C. and Gay CC (1995): Veterinary Medicine. 8th edition London; 563-574.

Raemy, A.; Meyian, M.; Casati, S.; Gaia, V.; Berchtold, B.; Boss, R.; Wyder, A. and Graber, H.U. (2013): Phenotypic and genotypic identification of Streptococci and related bacteria isolated from bovine intramammary infections. A.V.S. 55:53-62.

Ranjan, R.; Gupta, M.K. and Singh, K.K. (2011): Study of bovine mastitis in different climatic conditions in Jharkhand, India, Veterinary World, 4 (5): 205-208.

Rosini, R.; Rinaudo, C.D.; Soriani, M.; Lauer, P.; Mora, M., et al. (2006): Identification of novel genomic islands coding for antigenic pilus-like structures in Streptococcus agalactiae. Mol. Microbiol 61: 126-141.

Sambrook, J.; Fritscgh, E.F. and Mentiates .(1989): Molecular coloning. A laboratory manual.Vol !., Cold spring Harbor Laboratory press, New York.

Sargeant- J.M; Scott, H.M.; Leslie, K.E.; Ireland, M.J. and Bashiri, .A. (1998): Clinical mastitis in dairy cattle in Ontario: frequency of occurrence

and bacteriological isolates. Can Vet J.; 39(1): 33–38.

Schalm, O. W.; Carroll, E. J. and Jain, N. C. (1971): Bovine mastitis. Lea and Febiger, Philadelphia.

Song, X.M.; Casal, J.P.; Bolton, A. and Potter, A.A. (2001): Surface-Expressed *mig* protein protects *Streptococcus dysgalactiae* against phagocytosis by bovine neutrophils. Infect. Immun., 69(10):6030-6037.

Srinivasan V.; Sawant A. A.; Gillespie B. E.; Headrick S. J.; Ceasaris L. and Oliver S. P. (2006) : Prevalence of enterotoxin and toxic shock syndrome toxin genes in Staphylococcus aureus isolated from milk of cows with mastitis. Foodborne Path. Dis; 3, 274–283. 10.1089/fpd.3.274.

Sukhnan and ,S.; Dogan, B.; Ayodele, M.O.; Zadoks, R.N.; Craver, M.P.J., et al. (2005):09-4917 Molecular Subtyping and Characterization of Bovine and Human Streptococcus agalactiae Isolates. J ClinMicrobiol 43: 1177-1186.

Tavakoli, M. and Pourtaghi, H. (2017): Molecular detection of virulence genes and multi-drug resistance patterns in Escherichia coli (STEC) in clinical bovine mastitis: Alborz province, Iran. Iranian Journal of Veterinary Research, Shiraz University. 18(3): 208-211.

Todhunter, D.; Smith, K. and Hogan J. (1995): Environmental streptococcal intramammary infections of the bovine mammary gland. J. Dairy Sci. 78:2366-2374.

Tranter, H. S. (1996): Foodborne illness: foodborne staphylococcal illness. Lancet 336: 1044-1046.

Turutoglu, H. A.; Salihoglu, H. &Ozturk, M. (1995): Aerobic agents that cause mastitis in dairy cows in the Marmara region. Pendik Veteriner Microbiyoloji Dergis,26(2) :125-235.

Winn, W.; Allen, S.; Janda, W.; Koneman, E.; Procop, G.; Schreckenberger, P. and Woods, G. (2006):Koneman'a color Atlas and Textbook of Diagnostic Microbiology. 6th Ed., Lippincott William of Wilkins: London and New York.

Yasin, W.M.; Sabiel, Y.A.; El-Gaddal, A.A. and Mansour, M.E. (2016) : Antimicrobial resistance of pathogenic bacteria isolated from mastitis cows in Khartoum State, Sudan. *British Microbiology Res J.* 17(6): 1-6.