Alexandria Journal of Veterinary Sciences

www.alexjvs.com

AJVS. Vol. 51(2): 112-117. November 2016

DOI: **10.5455/ajvs.204220**

Incidence of Coagulase Negative Staphylococcus Isolated from Mastitis Cows and Human Contact

Helmy A. Torky and Samaa M. Abu Tabeikh

Microbiology Dept., Fac. Of Vet. Med. Alex. Univ.

Key words:
Staphylococcus aureus, isolation, PCR.

Correspondence to:

Abstract

This study aimed to throw the light on the incidence of coagulase negative staphylococcus (CNS) in clinical mastitis and its surrounded people and if there is a relationships between the incidence of CNS in mastitic cows and in surrounded peoples. The samples of human and animals collected from the Talaat Mustafa dairy farm located in Noubareia Alexandria Egypt at Cairo-Alexandria Desert Road (Private farm) and the worker act in this farm. In this study, a total number of 50 lactating Fresian cows were investigated for clinical mastitis. The pharyngeal swabs (50 samples) collected from the farmers that closely related and intact with the cattle. The age of these individuals ranged from 15 – 60 years for determination the relationships between the bacteria isolated from the milk and human. The number of pharyngeal swabs samples were 50 samples. The milk samples were examined bacteriologically: twenty out of 50 samples (40%) were positive for staphylococcus. Eigt out of 20 samples (40%) were coagulase positive staphylococcus while 12 out of 20 samples (60%) were coagulase negative staphylococcus. Fifteen out of 50 (30%) examined human samples were positive for staphylococcus. Seven out of 15 (70%) of samples were coagulase positive while 8 out of 15 (53%) of samples were coagulase negative staphylococcus. The coagulase negative staphylococcus isolates were highly sensitive to ciprofloxacin and amikacin while intermediate sensitive for ampicillin/fnlbacterin and gentamycin and the resistant to amoxicillin and trimethoprime+ sulphamethaxol.

1. INTRODUCTION

Raw milk can contain a variety of disease-causing pathogens, as demonstrated by numerous scientific studies. These studies, along with numerous food borne outbreaks, clearly demonstrate the risk associated with drinking raw milk. Pasteurization effectively kills raw milk pathogens without any significant impact on milk nutritional quality. Identification of bacterial pathogens in milk from cows with mastitis is the definitive diagnosis of mastitis infections. It also provides information important for prevention and control of the disease. In most clinical laboratories, identification methods are based on microbiological culture of milk and biochemical identification of bacterial isolates recovered. (Phuektes, et al., 2001). Coagulase-positive (CoPS) and coagulase-negative (CoNS) staphylococci are normal commensals of the skin and mucosa, but are also opportunist pathogens. Meticillin-resistant (MR) and multidrug-resistant (MDR) isolates are increasing in human and veterinary healthcare. Healthy humans and other animals harbour a variety of staphylococci, including MR-CoPS and MR-CoNS. (Schmid et al., 2014). Coagulase negative staphylococci (CNS) have attracted increasing interest as they have been isolated from mastitis in dairy animals, they are potential zoonotic pathogens, and they have the capability to produce enterotoxins in food (El-Jakee et al. 2013;

Davis et al. 2013 and Podkowik et al. 2013). Another may produce biofilms. In the natural environment, this fact may change the potential of the described microorganisms to cause an infection. Biofilm-related genes such as *icaA* or *bap* are involved in cell aggregation and accumulation of the components of the biofilm. The observed differences between the prevalence of the *ica* gene have given rise to discrimination between virulent and a virulent strains (Percival and Knottenbelt 2011).

Biofilm formation is well-documented in staphylococci. It has been studied in humans with ocular infection. Murugan et al. have isolated multi-drug resistant *Staphylococcus* spp. from clinical cases of conjunctivitis and have confirmed that approximately 90 % of them can produce biofilms (Murugan et al. 2010).

Biofilms are also implicated in the pathogenesis of acute conjunctivitis related to punctal plugs (Yokoi et al. 2000). In a study by Hou et al. (2012), 28.1 % of investigated CNS bacterial strains were found to be positive using the MTP test and 40.63 % were classified as *icaA* gene carriers. The authors of the mentioned study have suggested that the observed *in vitro* biofilm production may correspond to the biofilm formation within the eye. In addition, Suzuki et al. have described a high prevalence of biofilm-forming *S. epidermidis* with the *icaA* gene (60 %) collected from the conjunctival sac in humans (Suzuki et al. 2005).

In conclusion, this study indicated that the prevalence of biofilm-producing strains among conjunctival isolates of CNS is relatively high, but prevalence alone should not be over-interpreted as critical for the pathogenesis of ocular problems (especially conjunctivitis) associated with CNS.

The sources of contamination of milk mostly from with different pathogens (Pereira et al., 2011 and Murphy and Boor, 2012) as E. coli and can transmitted to the milk during milking of cows. The exterior of the cow's udder and teats can contribute microorganisms that are naturally associated with the skin of the animal as well as microorganisms that are derived from the environment in which the cow is housed and milked. (Bramley and McKinnon, 1990).

1.Microscopic

factor that should be taken into account is that CNS

Cleaning and sanitizing procedures can influence the degree and type of microbial growth on milk contact surfaces by leaving behind milk residues that support growth as well as by setting up conditions that might select for specific microbial groups. (Thomas et al., 1966).

The degree of cleanliness of the milking system probably influences the total bulk milk bacteria count as much as, if not more than, any other factor (Olson and Mocquat, 1980). Water used on the farm might also be a source.

The most important bacterial isolates that can be transmitted from the animals through the milk to human and vice versa includes Staphylococci that, causes mastitis in animals and pharangitis in human (Garicia-Palomo and Augeri (2000).

Using of polymerase chain reaction (PCR) : For identification of the virulence of bacterial isolates that identified by the ordinary identification methods depending on the methods implied by (Karahan and Cetinkaya, 2006 and Morandi et al,.2007)

So this study aimed to throw the light on the incidence of coagulase negative staphylococcus (CNS) in clinical mastitis and its surrounded people and if there is a relationships between the incidence of CNS in mastitic cows and in surrounded peoples.

MATERIALS AND METHODS

A- samples:

Human and animals collected from the Talaat Mustafa dairy farm located in Noubareia Alexandria Egypt at Cairo-Alexandria Desert Road (Private farm) and the worker act in this farm. This samples were examined for mastitis by collecting mastitic milk
In this study a total number of 50 lactating Fresian cows were investigated for clinical mastitis.

B. Human pharyngeal swabs: The pharyngeal swabs collected from the farmers that closely related and intact with the cattle. The age of this individuals ranged from 15 – 60 years for determination the relationships between the bacteria isolated from the milk and human. The number of pharyngeal swaps samples 50 samples.

2.examination of the isolates: shape of staphylocci: grape like clusters

C. Biochemical identification : : (Quinn et al ,1994) The identification of the isolates were made using the tests of Catalase test, oxidase test, coagulase test, urease test, oxidation fermentation test (Quinn et al., 2002).

E-Antibiotic sensitivity test: according to *Zaky (2009):* For detection of the most antibiotic that affect and destruct the bacterial agents or the bacterial resist this antibiotics and this done through cultivation of the isolates on Muller's Hinton Agar then addition of suitable antibiotics .

F-Data analysis and statistics

The statistical analysis were made for detection of the significance of the incidence of isolated microorganisms in milk of cows and buffaloes using Chi^2-test according to (SAS, 2004).

3. RESULTS AND DISCUSSION

Coagulase-negative staphylococci (CoNS) belong to saprophytic microbiota on the skin and mucous membranes of warm-blooded animals and humans, but are also isolated from foodstuffs such as meat, cheese, and milk. In other circumstances, some CoNS can act as pathogens. Thus, the presence of CoNS may not be an immediate danger to public health, but can become a risk factor. In particular antibiotic-resistant genes could be transferred to other potentially pathogenic microorganisms. Furthermore, CoNS are known to be strong biofilm producers and this is also a risk factor for public health. (Artini et al., 2015).

The results cleared in Table (1) indicated that, there is significant ($P < 0.01$) differences of the incidences of the number of samples gave positive staphylococcus and those gave negative results to staphylococcus among examined samples. The percentage of positive staphylococcus samples reached to 20 samples (40 %) and those gave negative results reached to 30 samples (60 %). Our results agreed with those of (Hamiroune et al., 2014) where they reported that, seventy percent of the milk analyzed was free from staphylococci and most of the bacteria identified were not pathogenic to consumers (coagulase- negative staphylococci); nevertheless, consuming fresh milk still presents a degree of risk.

Table (1): Incidence of staphylococcus organisms among examined mastitic milk.

Item	Number	Percent
Number of samples	50	100
Samples +ve to staphylococcus organisms	20	40
Number of negative samples to staphylococcus organisms	30	60

$Chi^2 = 19.33^*$ * = Significant at ($P < 0.05$).

Sensitivity of isolated staphylococcus organisms isolated from mastitic milk to coagulase test:

The results observed in Table (2) cleared that, that there is a significant differences ($P < 0.01$) of the incidences of coagulase +ve and –ve staphylococci. The number of Staphylococcus isolates reached to 20 isolates 8 (40 %) of them showed coagulase +ve staphylococci and 12 (60 %) of them showed coagulase negative staphylococci. Our results agreed with those of (Becker et al., 2014) where they reported that, the incidence of coagulase –ve staphylococci in mastitic milk higher than that of coagulase +ve staphylococci.

Table (2): Sensitivity of isolated staphylococcus organisms isolated from mastitis milk to coagulase test.

Item	Number	Percent
Number of stapgylococcus isolates	20	100
Coagulase +ve staphylococcus	8	40
Coagulase –ve staphylococcus	12	60

$Chi^2 = 22.44^*$ * = Significant at ($P < 0.05$).

Our results agreed with those of (Becker et al., 2014), where they reported that, the surrounded people to mastitic animals can suffer from respiratory manifestations conditions tat differ in its severity according to the types of organisms that causes in infection and the age of individual.

Our results agreed with those of (Płoneczka-Janeczko et al., 2014) where they reported that, the pharyngeal secretion colour can changed from clear or yellow and green colour according to the type of microorganisms.

Table (3) cleared that, there is a significant ($P < 0.01$) differences of the incidences of staphylococcus organism among examined samples (50 sample). The number of samples gave +ve results to staphylococci reached to 15 (30 %) , while that gave –ve

staphylococci results reached to 35 (70 %) of examined samples.

Table (3): Incidence of staphylococcus organisms among examined pharyngeal swabs collected from the surrounded people.

Item	Number	Percent
Number of samples	50	100
Samples +ve to staphylococcus organisms	15	30
Number of negative samples to staphylococcus organisms	35	70

$Chi^2 = 17.22^*$ * = Significant at ($P < 0.05$).

Table (4) cleared that, there is a significant ($P < 0.01$) differences in incidences of coagulase +ve and coagulase –ve staphylococci among examined samples. The results indicated that, the incidences of coagulase =ve staphylococci reached to 7 (46.67) and the incidences of coagulase –ve staphylococci reached to 8 (53.33 %).

Our results agreed with those of (Schmid et al., 2014) where they observed that, the incidence of coagulase –ve staphylococci in pharyngeal swab higher than that of coagulase –ve staphylococci.

Table (4): Sensitivity of isolated staphylococcus organisms isolated from pharyngeal swab to coagulase test.

Item	Number	Percent
Number of staphylococcus isolates	15	100
Coagulase +ve staphylococcus	7	46.67
Coagulase –ve staphylococcus	8	53.33

$Chi^2 = 19.22^{**}$ ** = Significant at ($P < 0.05$).

The results observed in Table (5) indicated that, the most sensitive and effective drug on coagulase negative staphylococci were ciprofloxacin, Amikin.

The intermediate effective drugs includes Ampicillin/fnlbactein and gentamycin.

The drug that showed no effect or resistant from coagulase negative staphylococci isolates includes amoxicillin, ampicillin, cefepime, caltriaxion. Amoxicillin, amoxicillin + clavenillic acid and trimethoprime + sulphamethaxol. Our results agreed with those of (De Visscher et al., 2013 and Schmidt et al., 2014) where they reported that, the amoxicillin, ampicillin of highly effective on coagulase negative staphylococci by they resist many drugs than the coagulase +ve staphylococci.

Table (5): Antibiotic sensitivity test.

Sample	Sxt	AMCB	CN10	AX25	E15	CRO50	FEP30	Ak30	SAM20	CIP5	AML10	AM10
1	R	R	S	R	I	R	R	S	S	S	R	R
2	R	R	I	R	I	R	R	S	S	S	R	R
3	R	R	S	R	I	R	R	I	I	S	R	R
4	R	I	S	I	S	R	R	S	I	S	I	I
5	R	R	I	R	R	R	R	S	I	S	R	R
6	R	R	I	R	R	R	R	S	I	S	R	R
7	R	R	S	R	R	R	R	S	I	S	R	R
8	R	R	I	R	R	R	R	S	I	S	R	R
9	R	R	I	I	R	R	I	S	S	S	R	R
10	R	R	I	R	R	R	R	S	I	S	R	I
11	R	R	I	R	R	R	R	S	I	S	R	R
12	R	R	I	I	R	R	R	S	I	S	R	R

1-E15 : Erythromythin 15 µg 2-AM10 : Ampicillin 10 µg 3-AML10 : Amoxicillin 10 µg
4-CN10 : Erythromycin 10 µg 5-CIP5 : Ciprofloxacin 5 µg 6-AMC30 : Amoxicillin + Clovinilic acid 7-SAM20: Ampicillin / fnlbacterin 10 8-CRO30 : Cltriaxon 30 9-Ax 25 : Amoxicillin 25
10-CEP25 : Cefoperazone 75 µg 11-FEP 30: Cefepime 30 µg 12-AK30 : Amikacin 30 µg
13-Ceftazidine 30: CAZ 30 µg 14 SXT : Trimethoprime + Sulphamethaxole.

It was concluded that, the incidence of coagulase negative staphylococci higher than
that coagulase positive staphylococci Coagulase-negative staphylococci (CoNS) belong to saprophytic microbiota on the skin and mucous membranes, its incidence in winter higher than that, of summer season

4. REFERENCES

Artini M, Cellini A, Papa R, Tilotta M, Scoarughi GL, Gazzola S, Fontana C, Tempera G,Cocconcelli PS, Selan L. (2015): Adhesive behaviour and virulence of coagulase negative staphylococciisolated from Italian cheeses. Int J Immunopathol Pharmacol. 2015 Aug |volume 3 Page No35

Bexiga R, Rato MG, Lemsaddek A, Semedo-Lemsaddek T, Carneiro C, Pereira H, Mellor DJ, Ellis KA, Vilela CL. (2014): Dynamics of bovine intramammary infections due to coagulase-negative staphylococci on four farms. J Dairy Res. 2014 May; page 81(2):208-214.

Bramley, A.J. and C.H. McKinnon. 1990. The microbiology of raw milk. pp. 163-208. In Dairy Microbiology, Vol. 1. Robinson, R.K. (ed.) Elsevier Science Publishers, London.

Davis MF, Cain CL, Brazil AM, et al. Two coagulase-negative staphylococci emerging as potential zoonotic pathogens: wolves in sheep's clothing? Front Microbiol. 2013; 4: 123.

De Visscher A, Haesebrouck F, Piepers S, Vanderhaeghen W, Supré K, Leroy F, Van Coillie E, De Vliegher S. (2013): Assessment of the suitability of mannitol salt agar for growing bovine-associated coagulase-negative staphylococci and its use under field conditions. Res Vet Sci. 2013 Oct;volume 95(2):page 347-51.

El-Jakee JK, Aref NE, Gomaa A, et al. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: an environmental hazard. Int J Vet Sci Med. 2013;1:74–78.

Garcia-Palomo D., and Augeri J. (2000): Osteomyelitis caused by Staphylococcus schleiferi and evidence for this Staphylococcus species by an automated identification system. J. Clin. Microbiol., 43: 2286-2290.

Hamiroune M, Berber A, Boubekeur S. (2014): Contribution to the study of staphylococcus contamination of cows' milk on a number of farms in Algiers: its impact on human health. Rev Sci Tech. 2014 Dec;33(3):1035-41, 1027-34.

Hou W, Sun X, Wang Z, et al. Biofilm-forming capacity of*Staphylococcus epidermidis*, *Staphylococcus aureus* and*Pseudomonas areuginosa* from ocular infection. Invest Ophtalmol Vis Sci. 2012; 53:5624–5631. doi: 10.1167/iovs.11-9114.

Karahan M., Cetinkaya B. (2006): Coagulase gene polymorphism detected by PCR in Staphylococcus aureus isolated from subclinical mastitis in Turkey Vet. J. 2006 Aug. 8

Morandi, S.; Brasca, M.; Lodi, R.; Germonesi, P.; Catiglioni, B. (2007): Detection of classical enterotoxins and identification of enterotoxin genes in S. aureus from milk and dairy products. Vet. Microbiol. 2007 Mar. 27; [Epub a head of print].

Murphy E, Spencer S, Young D, et al. (2011): MRSA colonisation and subsequent risk of infection despite effective eradication in orthopaedic elective surgery. J Bone Joint Surg (Br) 2011;93:548–551.

Murugan K, Usha M, Malathi P, et al. Biofilm forming multi drug resistant Staphylococcus spp. among patients with conjunctivitis.Pol J Microbiol. 2010;59:233–239.

Olson, J.C. Jr., and G. Mocquat. 1980. Milk and Milk Products. p. 470. In Microbial Ecology of Foods. Vol. II. J.H. Silliker, R.P. Elliott. A.C. Baird-Parker, F.L. Bryan, J.H. Christion, D.S. Clark, J.C. Olson, and T.A. Roberts (eds.). Academic Press, New York, NY.

Phuektes P., Mansell P. D. and Browning G. F. (2001): Multiplex Polymerase Chain Reaction Assay for Simultaneous Detection of Staphylococcus aureus and Streptococcal Causes of Bovine Mastitis. j. Dairy Sci. 84:1140-1148

Percival SL, Knottenbelt DC. Cochrane CA. (2011): Biofilms and veterinary medicine. Berlin: Springer; 2011.

Pereira RV, Santos TM, Bicalho ML, Caixeta LS, Machado VS, Bicalho RC. (2011): Antimicrobial resistance and prevalence of virulence factor genes in fecal Escherichia coli of Holstein calves fed milk with and without antimicrobials. J Dairy Sci. 2011 Sep;94(9):4556-65.

Płoneczka-Janeczko K[1], Lis P, Bierowiec K, Rypuła K, Chorbiński P. (2014): Identification of bap and icaA genes involved in biofilm formation incoagulase negative staphylococci isolated from feline conjunctiva. Vet Res Commun. 2014 Dec;38(4):337-46.

Podkowik M, Park JY, Seo KS, et al. 2013.Enterotoxygenic potential of coagulase-negative staphylococci. Int J Food Microbiol.163:34–page 40.

SAS, 2004: Statistical analysis system. SAS User's Guide. SAS Incorporation Institute.

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

Schmidt T[1], Kock MM[2], Ehlers MM[2]. (2015): Diversity and antimicrobial susceptibility profiling of staphylococci isolated from bovine mastitis cases and close human contacts. J Dairy Sci. 2015 Jul 15.

Schmidt, V. M.; Williams, N. J.; Pinchbeck, G.; Corless, C. E.; Shaw, S.; McEwan, N. ; Dawson, S. and Nuttall, T. (2014): Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. BMC Vet Res. 2014; 10: 17. Published online 2014 Jan 14.

Suzuki T, Kawamura Y, Uno T, et al. Prevalence of Staphylococcus epidermidis strains with biofilm-forming ability in isolates from conjunctiva and facial skin. Am J Ophtalmol.2005; 140:844–page 850.

Thomas S.B., R.G. Druce and K.P. King. (1966): The microflora of poorly cleansed farm dairy equipment. J. Appl. Bacteriol.volume 29:409.

Yokoi N, Okada K, Suquita J, et al. Acute conjunctivitis associated with biofilm formation on a punctal plug. Jpn J. Ophtalmol. 2000; 44:559–page 560.

Zaky, M. M. M. (2009): Occurrence of Antibiotic-Resistant and Plasmid DNA Harbouring Bacterial Pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt Research Journal of Microbiology Year: 2009 | Volume: 4 | Issue: 2 | Page No18.