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Full Length Article

Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard☆

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Abstract In Egypt, knowledge about the coagulase negative staphylococci (CNS) involved in mastitic animals is limited. CNS have emerged to be pathogens causing intramammary infections in Egyptian dairy herds. Therefore, the current study was conducted to investigate the occurrence of CNS in dairy ruminants (cattle, buffaloes, sheep and goats). A total of 884 quarter milk samples were investigated to study the prevalence of CNS among mastitic and subclinically mastitic cows, buffalo–cows, ewes and does in Egypt. Identification of the isolates was achieved using API staph test and polymerase chain reaction (PCR). CNS were isolated from the examined subclinical mastitic cattle, buffaloes, sheep and goats with percentages of 16.6%, 59.4%, 50% and 55.6%, respectively. *Staphylococcus xylosus*, *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus chromogenes*, *Staphylococcus lentus*, *Staphylococcus lugdunensis* and *Staphylococcus simulans* were identified as CNS that recovered from the examined milk samples. The CNS as mastitis-causing agents could not be neglected as they can cause substantial economic losses.

1. Introduction

Mastitis continues to cause a huge economic burden for the dairy industry. To date, more than 50 *Staphylococcus* species and subspecies have been characterized to cause staphylococcal mastitis. The genus is divided into coagulase positive staphylococci and coagulase negative staphylococci (CNS) based on their ability to coagulate plasma [1]. The major bacterial agents incriminated in intramammary infection (IMI) are *Staphylococcus aureus*, streptococci, coliforms, *Mannheimia* species, *Arcanobacterium pyogenes*, *Pasteurella* species, etc.

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While coagulase-negative staphylococci and *Corynebacterium bovis* were considered as minor pathogens causing mastitis. These kinds of pathogens were considered to be minor based on the fact that the minor pathogens are not reported quantitatively from each laboratory diagnosing mastitis [2].

CNS have become the most common bacterial pathogens isolated from milk samples in many countries causing bovine intramammary infections [3,4] and could be described as emerging mastitis pathogens [1]. They are opportunists and adhere to metal devices to produce a protective biofilm. The ability to produce biofilm enables CNS to persist on milking equipment as well as on the milker's hands, which serves a major source of staphylococcal spread [5]. CNS have traditionally been considered to be normal skin microbiota, which as opportunistic bacteria can cause mastitis. In dairy farms that have successfully controlled mastitis caused by *S. aureus* and *Streptococcus agalactiae*, CNS became the frequently associated cause of bovine mastitis instead [6]. The knowledge concerning CNS involved in mastitis in Egypt is still very limited. Therefore, the aim of the current investigation is to study the role of CNS among mastitic cows, buffaloes, sheep and goats in Egypt.

2. Materials and methods

2.1. Sampling

A total of 884 quarter milk samples were collected from 158 cows, 43 buffaloes, 20 sheep, and 20 goats in different dairy farms in Egypt as shown in Table 1. A clinical examination of each udder (4 quarters in cows and buffaloes–2 halves in sheep and goats) was conducted in order to detect the animals with signs of clinical mastitis. Each teat was thoroughly washed and then disinfected. The first few streams of foremilk were discarded and the middle stream of each milk sample was aseptically collected into sterile containers [7]. Samples collected from apparently normal quarters of the udder were subjected to California Mastitis Test (CMT) using mastitis indicator test kit (Frieso-test) obtained from Impfstoff work Friesoythe Gmbh-Germany as a field test recommended by the American Public Health Association [8] to detect the incidence of subclinical mastitis.

2.2. Isolation and identification of CNS isolates

Each milk sample was incubated for 18–24 h at 37 °C. Preliminary incubation of milk samples was used to isolate the colony-forming units of the organisms below the detection limit in milk samples. The present study was focus on isolation and identification of CNS organisms [9,10]. A loopfull from each sample was cultured on mannitol salt agar (Oxoid Ltd., Hampshire, UK) and 5% sheep blood agar. All plates were incubated at 37 °C for 18–24 h and examined for bacterial growth. Suspected colonies were analyzed for: hemolytic pattern on sheep blood agar, lecithinase activity on Baird Parker medium (Oxoid Ltd., Hampshire, UK), supplemented with egg yolk tellurite emulsion [11,12]. Also, API-Staph Kit (bioMérieux SA, l'Etoile, France) was used for identification of CNS isolates following the instructions of kit's insert and then the strips were read by the mini API instrument and associated software.

Table 1 Types and numbers of the collected milk samples.

Animal species	No. of examined animals	Apparently healthy quarters	Clinical mastitic quarters
Cow	158	620	12
Buffaloes	43	168	4
Sheep	20	40	0
Goat	20	40	0
Total	241	868	16

2.3. PCR for identification of CNS isolates

A rapid procedure was used to prepare template DNA as described previously [13]. Multiplex PCR assay was performed utilizing two sets of primers: 16SrRNA specific primers for *Staphylococcus* genus (16SrRNA f – 5′-GTA GGT GGC AAG CGTTAT CC-3′ and 16SrRNA r – 5′-CGC ACA TCA GCG TCA G-3′) [14] which amplified 228 bp product and *S. aureus* specific primers (Nuc 1 – 5′-GCGATTGATGGT GATACGGTT-3′ and Nuc 2 – 5′-AGCCAAGCCTTGAC-GAACTAAAGC-3′) [15] to amplify 279 bp products. The PCR assay was established using a total volume of 25 μl reaction mixtures contained 5 μl of DNA as template, 20 pmol of each primer and 1× of PCR master mix (5× Taq Master/High yield, Jena Bioscience). The amplification cycles were carried out in a PT-100 Thermocycler (MJ Research, USA). Reaction conditions were optimized to be 94 °C for 4 min. as initial denaturation, followed by 35 cycles of 94 °C for 60 s, 55 °C for 60 s and 72 °C for 60 s. A final extension step at 72 °C for 10 min. was followed. Positive DNA isolated from *S. aureus* strain ATCC# 25923 and negative control (no template) were included in each PCR run to ensure no cross contamination or amplification failure due to presence of inhibitors. The PCR product was visualized on a 1.5% agarose gel by using UV trans-illuminator (UVP, Fisher Scientific) [16]. To assure that the amplification products were of the expected size, a 100 bp DNA ladder (Promega) was run simultaneously as a marker.

3. Results

3.1. Identification of CNS isolates

All isolates were Gram positive non-spore forming cocci, arranged in clusters, and catalase positive. The isolates were: *Staphylococcus lugdunensis*, *Staphylococcus xylosus*, *Staphylococcus cohnii*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, *Staphylococcus chromogenes*, *Staphylococcus lentus* and *Staphylococcus simulans* that have been identified biochemically by API-Staph Kits. By using PCR, it was clear that staphylococci specific genus primer (16srRNA) generated 228 bp amplicon from all CNS isolates, while the selected *S. aureus* primer (Nuc) could not generated 279 bp amplicon from all CNS isolates as shown in Fig. 1. So, amplification of both 228 and 279 bp bands was recorded among *S. aureus* ATCC# 25923 (standard strain).

3.2. Occurrence of CNS among the examined cows and buffaloes

It is clear from Table 2 that CNS could not be detected from clinical mastitic cow and buffalo samples. On the other hand,

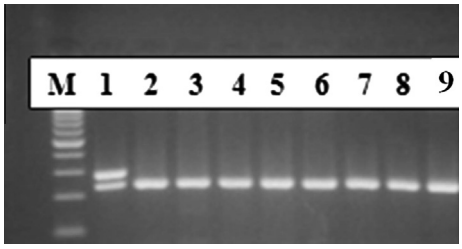

Fig. 1 The multiplex PCR assay to detect *Staphylococcus* isolates. M: 100 bp DNA ladder (Fermentas). Lane 1: *S. aureus* ATCC 25923 reference strain (228 and 279 bp amplified bands). Lanes 2–9: CNS isolates (228 bp only).

S. lugdunensis, *S. xylosus*, *S. cohnii*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus* and *S. lentus* were detected in subclinical mastitic cow samples. Among apparently hea
lthy cases (Negative for CMT), *S. xylosus*, *S. cohnii*, *S. hominis*, and *S. chromogenes* were identified. Among subclinical mastitic buffalo samples, *S. xylosus*, *S. cohnii*, *S. hominis*, *S. lugdunensis* and *S. simulans* were detected and among apparently healthy cases, *S. xylosus*, *S. cohnii*, *S. hominis*, and *S. lugdunensis* were identified. *S. aureus* was detected from negative CMT, subclinical mastitis and clinical mastitis cow and buffalo samples with different rates as shown in Table 2.

3.3. Occurrence of CNS among the examined sheep and goat

S. hominis, *S. lugdunensis* and *S. saprophyticus* were detected in subclinical mastitic goat samples, while *S. cohnii* and *S. lugdunensis* were detected in subclinical mastitic sheep samples. On the other hand, *S. aureus* was detected from negative CMT and subclinical mastitic goat and sheep samples with different rates as shown in Table 3.

4. Discussion

The importance of coagulase-negative staphylococci (CNS) has increased and they have become the predominant pathogens isolated from subclinical mastitis in several countries [17–19]. In the present investigation, the occurrence of CNS among 884 quarter milk samples collected from mastitic cows, buffaloes, sheep and goat was studied.

The present data illustrated that *S. aureus* was the predominant isolate recovered from the examined cow, buffalo, sheep and goat samples. The predominance of *S. aureus* among the mastitis causing agents was recorded earlier in cattle and sheep [5]. The high rates of *S. aureus* may be due to that the rates were calculated according to the isolates recovered from each quarter (4 among bovine and 2 among sheep and goat) rather than from each animal. In addition, *S. aureus* is localized inside and/or outside the udder and causes both clinical and subclinical mastitis, however, the concerning samples may give negative results with CMT. False positive and false negative reactions still occur with CMT and there is misclassification biases as well [2].

CNS were detected from clinically and subclinical mastitic cows, buffaloes, sheep and goat in a relatively high rates (Tables 2 and 3). It is quite common for CNS to be recovered from about 15% to 20% of bovine milk samples obtained from cows experiencing subclinical and clinical mastitis [20–22]. Many studies in different regions worldwide have been investigated the prevalence of CNS causing mastitis. The occurrence of intramammary infections with CNS was reported in Finland where CNS were isolated from 50% of the quarters positive for bacterial growth in a nationwide survey [23]. In a similar study that was conducted in Norway, the occurrence of CNS was 16% [24], while in Germany, CNS were isolated from 9% of the quarter milk samples in a total of 80 dairy herds [17]. In two dairy herds in Canada, CNS were the most

Table 2 The prevalence of CNS among the examined cows and buffaloes.

Isolated bacteria	Cow milk samples (N = 632)						Buffalo milk samples (N = 172)					
	Negative CMT (N = 161)		Subclinical mastitic samples (N = 459)		Clinical mastitic samples (N = 12)		Negative CMT (N = 40)		Subclinical mastitic samples (N = 128)		Clinical mastitic samples (N = 4)	
	n	(n/N) %	n	(n/N) %	n	(n/N) %	n	(n/N) %	n	(n/N) %	n	(n/N) %
S. aureus	66	41	235	51.2	12	100	6	15	47	36.7	4	100
S. xylosus	17	10.6	30	6.5	–	0	2	5	19	14.8	–	0
S. cohnii	4	2.5	5	1.1	–	0	4	10	5	3.9	–	0
S. haemolyticus	–	0	1	0.2	–	0	–	0	–	0	–	0
S. hominis	10	6.2	19	4.1	–	0	4	10	12	9.4	–	0
S. saprophyticus	–	0	5	1.1	–	0	–	0	–	0	–	0
S. chromogens	4	2.5	0	0	–	0	–	0	–	0	–	0
S. lentus	–	0	4	0.9	–	0	–	0	–	0	–	0
S. lugdunensis	–	0	–	0	–	0	4	10	6	4.7	–	0
S. simulans	–	0	–	0	–	0	–	0	4	3.1	–	0
S. aureus and *S. lugdunensis*	0	0	12	2.6	–	0	–	0	18	14.1	–	0
S. aureus and *S. xylosus*	–	0	–	0	–	0	–	0	12	9.4	–	0
No bacterial growth	60	37.3	148	32.3	–	0	20	50	5	3.9	–	0
Total CNS	35	21.7	76	16.6	–	0	14	35	76	59.4	–	0

N = Number of total samples.
n = Number of positive samples.
% was calculated according to the number of examined samples.
CMT = California mastitis test.

Table 3 The prevalence of CNS among the examined goat and sheep.

Bacterial species	Goat milk samples ($N = 40$)				Sheep milk samples ($N = 40$)			
	Negative CMT ($N = 4$)		Subclinical mastitic samples ($N = 36$)		Negative CMT ($N = 16$)		Subclinical mastitic samples ($N = 24$)	
	n	(n/N) %	n	(n/N) %	n	(n/N) %	n	(n/N) %
S. aureus	0	0	16	44.4	8	50	12	50
S. cohnii	0	0	0	0	0	0	8	33.3
S. hominis	0	0	4	11.1	0	0	0	0
S. lugdunensis	0	0	12	33.3	0	0	4	16.7
S. saprophyticus	0	0	4	11.1	0	0	0	0
No bacterial growth	4	100	0	0	8	50	0	0
Total CNS	0	0	20	55.6	0	0	12	50

N = Number of total samples.
n = Number of positive samples.
% was calculated according to the number of examined samples.
CMT = California mastitis test.

common bacteria (51%) causing intramammary infection (IMI) at drying off period [19].

The results of the current investigation reported that CNS is generally high in subclinical mastitic samples, but low in samples from animals with clinical mastitis. Reports on the clinical characteristics of coagulase negative staphylococcal mastitis are scarce as CNS have been ignored in many studies on clinical mastitis, so that CNS infection mostly remained subclinical [1]. Whereas, there was a study has been occurred in Finland on clinical characteristics of bovine mastitis caused by CNS [25], in which approximately half of the mastitis cases recorded were clinical, and the majority of cases showed mild clinical signs. The authors added that the severity and persistence of intramammary infection were unaffected by CNS species.

The identified species of CNS from the examined milk samples in the current study were *S. xylosus*, *S. cohnii*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. chromogenes*, *S. lentus*, *S. lugdunensis* and *S. simulans*. In concordance to our results, many articles have reported the common staphylococci isolated from mastitic herds were *S. chromogenes*, *S. hyicus*, *S. simulans*, *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. xylosus*, *Staphylococcus warneri*, *Staphylococcus sciuri*, *Staphylococcus capitis*, *S. saprophyticus* and *S. lentus* [26–29] and they assured that the environment was found as a reservoir, suggesting that IMI with such bacterial species is possibly considered as an environmental hazard.

There are moderate increases in the mean of somatic cell count (SCC) in milk samples infected with CNS (unpublished data). SCC in quarters infected with CNS is rather low compared with infections caused by *S. aureus* [30]. Besides increasing SSC, CNS infections also can result in decreased milk quality, damage of the udder tissue and decrease milk production [1]. In general, mastitis leads to changes in milk composition and reduces its quality. If treated with antibiotics, the existence of antibiotic in the milk interferes with the process of dairy products and may cause health problems to a consumer which is considered a public health hazard. On the other hand, control measure of the subclinical mastitis is much more important than clinical cases because most of subclinical mastitic cows are reservoirs for pathogens that may lead to spread of infection to other neighboring cows.

It could be concluded that, CNS are emerging as important minor mastitis pathogens in Egyptian dairy animals. Such species can cause substantial economic losses resulting in decreased milk production. This reflects the environmental hazard and therefore, udder health must be followed up and control of intra-mammary infections is consequently of the greatest importance for dairy farms. Further investigations should be occurred on the epidemiology of CNS-causing mastitis and more reliable identification methods would be beneficial for dairy industry.

References

[1] Pyorala S, Taponen S. Coagulase-negative staphylococci-emerging mastitis pathogens. Vet Microbiol 2009;134:3–8.
[2] Dingwell RT, Leslie KE, Schukken YH, Sargeant JM, Timms LL. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J 2003;44(5):413–6.
[3] Radostits MO, Gay CC, Blood DC, Hinchcliff KW. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. W.B. Saunders Company Ltd; 2000.
[4] Simojoki H, Orro T, Taponen S, Pyörälä S. Host response in bovine mastitis experimentally induced with *Staphylococcus chromogenes*. Vet Microbiol 2009;134:95–9.
[5] Pilipčincová I, Bhide M, Dudriková E, Trávniček M. Genotypic characterization of coagulase-negative staphylococci isolated from sheep milk in Slovakia. Acta Vet Brno 2010;79:269–75.
[6] Ruegg PL. The quest for the perfect test: phenotypic versus genotypic identification of coagulase-negative staphylococci associated with bovine mastitis. Vet Microbiol 2009;134:15–9.
[7] Blood DC, Handerson JA. Veterinary medicine. 3rd ed. London: Baillier Tinfall and Gassel; 1986.
[8] American Public Health Association "APHA". Standard methods for examination of dairy products. 16th ed. New York: American Public Health Association, Inc; 1992.
[9] Wilson CD, Slavin G. Remove from marked Records A direct culture method for the examination of milk samples. J Comp Pathol 1950;60(3):230–4.
[10] Ledford RA, Senyk GF, Kotsides E, Wolff ET. Preliminary incubation method for detection of coliforms in commercially processed milk samples. J Dairy Sci 1983;66(8):1607–10.
[11] Forbes BA, Sahm DF, Weissfeld AS. Baily and Scott's diagnostic microbiology. 12th ed. St. Louis, Missouri: Mosby Elsevier; 2007.

[12] Abdel-moein K, El-Hariri M, Samir A. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen of pets in Egypt with a public health burden. Transbound Emerg Dis 2012;59(4):331–5.
[13] Reischl U, Pulz M, Ehret W, Wolf H. PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. BioTechniques 1994;17:844–5.
[14] Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various food in Portugal. Food Microbiol 2009;26(3):278–82.
[15] Zhang K, Sparling J, Chow BL, Elsayed S, Hussain Z, Church DL, et al. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J Clin Microbiol 2004;42:4947–55.
[16] Sambrook J, Fritsch FE, Maniatis J. Molecular cloning: a laboratory manual. 2nd ed. Philadelphia: Cold Spring Harbor Laboratory press; 1989.
[17] Tenhagen BA, Koster G, Wallmann J, Heuwieser W. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J Dairy Sci 2006;89:2542–51.
[18] Koivula M, Mantysaari EA, Pitkala A, Pyorala S. Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland. Acta Agric Scand A 2007;57:89–96.
[19] Lim GH, Leslie KE, Kelton DF, Duffield TF, Timms LL, Dingwell RT. Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period. J Dairy Sci 2007;90:1289–300.
[20] Makovec JA, Ruegg PL. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci 2003;86:3466–72.
[21] Hoe FG, Ruegg PL. Relationship between antimicrobial susceptibility of clinical mastitis pathogens and treatment outcome in cows. J Am Vet Med Assoc 2005;227:1461–8.
[22] Pol M, Ruegg PL. Relationship between antimicrobial drug usage and antimicrobial susceptibility of Gram-positive mastitis pathogens. J Dairy Sci 2007;90:262–73.
[23] Pitkala A, Haveri M, Pyorala S, Myllys V, Honkanen-Buzalski T. Bovine mastitis in Finland 2001 – prevalence, distribution of bacteria, and antimicrobial resistance. J Dairy Sci 2004;87:2433–41.
[24] Osteras O, Solverod L, Reksen O. Milk culture results in a large Norwegian survey effect of season, parity, days in milk, resistance, and clustering. J Dairy Sci 2006;89:1010–23.
[25] Taponen S, Simojoki H, Haveri M, Larsen HD, Pyorala S. Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Vet Microbiol 2006;115:199–207.
[26] Sawanta AA, Gillespiea BE, Oliver SP. Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. Vet Microbiol 2009;134:73–81.
[27] Thorberg BM, Danielsson-Tham ML, Emanuelson U, Persson Waller K. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J Dairy Sci 2009;92(10):4962–70.
[28] Hogan JS, White DG, Pankey JW. Effects of teat dipping on intramammary infections by staphylococci other than *Staphylococcus aureus*. J Dairy Sci 1987;70:873–9.
[29] Piessens V, Van Coillie E, Verbist B, Supré K, Braem G, Van Nuffel A, et al. Distribution of coagulase-negative *Staphylococcus* species from milk and environment of dairy cows differs between herds. J Dairy Sci 2011;94:2933–44.
[30] Brinda M, Herman V, Fodor I. Phenotypic characterization of coagulase-negative staphylococci isolated from mastitic milk cows, vol. XLIII. Timisoara: Lucrari Stiinifice Medicina Veterinara; 2010.