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CHARACTERIZATION OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM MASTITIC ANIMALS

By

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ABSTRACT

Coagulase negative staphylococci (CNS) are the predominant pathogens causing intramammary infections (IMI) in dairy herds. A total of 804 milk samples were investigated to study the prevalence of staphylococci among dairy cows (n=632) and buffaloes (n=172) in Egypt. Phenotypic identification of the isolates was achieved by using conventional identification methods, API staph ID test and PCR. *S. aureus* was isolated from the examined cow and buffaloes in pure culture with incidence of 49.5 and 33.1%, respectively. While CNS were isolated from the examined cow and buffaloes in pure cultures with incidence of 15.7 and 34.9 %, respectively. *S. cohnii, S. lugdunensis, S. saprophyticus, S. xylosus, S. hominis, S. simulans* and *S. lentus,* were isolated from the collected samples and associated with a slight to moderate increase in the number of somatic cells in milk. The significance of CNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents.

Keywords:

Cow, buffalo, mastitis, *S. aureus*, coagulase negative staphylococci (CNS), polymerase chain reaction (PCR), antibiotic.

INTRODUCTION

Mastitis continues to cause a huge economic burden to the dairy industry. To date, more than 50 *Staphylococcus* species and subspecies have been characterized. The genus is divided into coagulase-positive staphylococci and coagulase negative staphylococci (CNS) based on their ability to coagulate plasma **(Taponen and Pyörälä, 2009).** CNS are a frequent cause of bovine intramammary infections (IMI), can cause infections in healthy host tissue and have become the most common bacteria isolated from milk samples in many countries **(Radostits *et al*., 2000, Taponen, 2008).** Recently **De Visscher *et al*. (2017)** recorded that coagulase-

negative staphylococci (CNS) have become the main pathogens causing bovine mastitis in recent years. They are opportunists and adhere to metal devices to produce a protective biofilm. The ability to produce biofilm enables CNS to persist on metal devices, milking equipments as well as on the miller's hands, which serve a major source of staphylococcal spread **(Ivana *et al.*, 2010).** CNS have traditionally been considered to be normal skin microbiota, which as opportunistic bacteria can cause mastitis. CNS were isolated from different body sites of cows, heifers and calves, from udder secretions and milk, and from the cows environment **(Taponen, 2008).** On farms that have successfully controlled mastitis caused by *S. aureus* and *S. agalactiae*, CNS is frequently associated with bovine mastitis **(Ruegg, 2009).** The knowledge on CNS species involved in mastitis in Egypt is still very limited. So the aim of this investigation was to characterize CNS isolated from mastitic cows and buffaloes.

MATERIAL AND METHODS

2.1. Sampling:

A total of 804 milk samples were investigated to study the prevalence of staphylococci among dairy cows (n=632) and buffaloes (n=172). The samples were aseptically collected into sterile tubes after cleaning and disinfection of each teat end **(Blood and Handerson, 1986)**. 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 (1992)** according to **Schalm *et al.* (1971)** to detect the incidence of subclinical mastitis. Also, somatic cell count (SCC) was measured automatically within 6 and 72 hours after sampling using a Bently Soma Count 150 (Bently U.S.A).

2.2. Bacteriological analysis and isolation procedures:

Each milk sample was incubated for 18-24 hours at 37 ºC and a loopfull from each sample was cultured on mannitol salt agar (Oxoid) and 5% sheep blood agar (SBA). All plates were incubated at 37 ºC for 18-24 hours and examined for bacterial growth. Suspected colonies were identified using routinely microbiological procedures and analyzed according to **Cruickshank *et al.* (1975)** and **Quinn *et al.* (2002).** API-Staph Kit bio Merieux was used for identification of CNS isolates and the strips were read by the mini API instrument and associated software.

2.3. Detection of CNS by PCR:

A rapid procedure was used to prepare template DNA as described by **Reischl *et al.* (1994).** Multiplex PCR assay was performed utilizing a 2 set 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') according to **Pereira *et al.* (2009)** which amplified 228 bp product and *S. aureus* specific primers
(Nuc1-5'- GCGATTGATGGTGATACGGTT-3' and Nuc 2 - 5'-AGCCAA GCCTT GACG AAC TAAAGC-3 ') as described by **Zhang *et al.* (2004)** to amplify 279 bp products.

2.4. β- lactamase activity and Antibiotic sensitivity test:

The β- lactamase activity among the CNS isolates was estimated using β-LACTA strip (Test-Line Ltd. Krizikova 68, 612 00 BRNO CZ.) according to **Livermore and Brown (2001).** Also on each isolate the inhibition zones of 11 antibiotics used in human and veterinary medicines were determined by disk diffusion method **(Finegold and Martin, 1982)**. The antibiotics (Oxoid) tested were amoxicillin (10µg/disk), amoxicillin + clavulanic acid (20+10 µg/disk), ampicillin (10 µg/disk), cloxacillin (5 µg/disk), erythromycin (15 µg/disk), florfenicol (30 µg/disk), gentamicin (10 µg/disk), neomycin (30 µg/disk), oxacillin (1µg/disk), oxytetracycline (30 µg/disk) and penicillin G (10 units /disk).

RESULTS

Identification of CNS isolates recovered from animals:

All isolates were Gram positive, catalase positive cocci, arranged in clusters and non-spore forming bacteria. The isolates were identified biochemically by API-Staph Kit bioMerieux as *S. lugdunensis*, *S. xylosus*, *S. cohnii*, *S. hominis*, *S. saprophyticus*, *S. lentus* and *S. simulans*.

Incidence of CNS among the examined cows and buffaloes:

It is clear from (Table 1) that, CNS could not be detected from clinical mastitic cow and buffalo samples. While in SCM samples, CNS were detected with incidence of 13.9 and 40.6 % among cow and buffalo samples, respectively. Among negative CMT cases CNS were identified from cow and buffalo samples with incidence of 21.7 and 20%, respectively.

Level of SCC among the examined CNS infected milk samples:

The correlation between CNS and SCC among the infected quarters was investigated as shown in (Table 2). It is clear that, the mean SCC was greater in milk samples positive for *S. aureus* when compared to those positive for CNS. The mean SCC was ≥ 500,000 cells/ml

among 62.9 and 36.8 % of *S. aureus* infected cow and buffalo samples respectively. While 27.3 and 20 % of CNS infected samples among the examined cow and buffalo, respectively had SCC ≥ 500,000 cells /ml. Furthermore, 38.4 and 36.7 % of CNS positive samples had SCC < 250,000 cells/ml among the examined cow and buffalo milk samples respectively.

Identification of *Staphylococcus* isolates using multiplex PCR:

Using PCR, as shown in Fig. (1) It is 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). Amplification of both 228 and 279 bp bands was recorded among *S. aureus* isolates and ATCC 25923 reference strain.

Characterization of the *Staphylococcus* isolates:

A total of 18 *S. aureus* isolates (11 from SCM cows and 7 from SCM buffaloes) and 45 CNS isolates (23 from SCM cows and 22 from SCM buffaloes) were investigated.

Pigmentation on mannitol salt agar:

As shown in Tables 3 and 4 and Fig. (2) it is clear that, 11 (47.8%), 10 (45.5%) CNS isolated from cows and buffaloes respectively had creamy colonies, while 12 (52.2%) and 12 (54.5%) CNS isolated from cows and buffaloes respectively had white colonies. *S. aureus* isolated from cows and buffaloes had golden yellow colonies.

Hemolysis on blood agar:

(Tables 5 and 6) and Fig. (3 and 4) illustrated that, 12 (52.2%) and 10 (45.5%) CNS isolated from cows and buffaloes respectively were α hemolysis and 11 (47.8%) and 12 (54.5%) CNS isolated from cows and buffaloes respectively were β hemolysis on blood agar. While 4 (36.4%) and 3 (42.9%) *S. aureus* isolated from cows and buffaloes respectively were α hemolysis and 7 (63.6%) and 4 (57.1%) *S. aureus* isolated from cows and buffaloes respectively were β hemolysis on blood agar.

β lactamase activity among the staphylococci isolates:

Using β -lacta strips, β lactamase activity was investigated among the CNS isolates as shown in (Tables 7) and Fig. (5). It is clear that, 22 (95.7%) and 14 (63.6%) CNS isolated from cows and buffaloes respectively were B lactamase positive.

While 9 (81.8%) and 6 (85.7%) *S. aureus* isolated from cows and buffaloes respectively were B lactamase positive.

Result of antibiotic sensitivity test among the staphylococci isolates:

(Tables 8-11) and Fig. (7) show the antibiotic resistant pattern of the isolates. It is clear that *S. aureus* isolates were sensitive to erythromycin, neomycin, oxytetracycline and florfenicol. Meanwhile the examined isolates were resistant to penicillin (100%), amoxicillin, ampicillin and oxacillin (94.4% each) and cloxacillin (88.9%).

Among CNS isolates they were sensitive to neomycin, florfenicol, gentamicin, erythromycin and oxytetracycline. Meanwhile the examined isolates were resistant to cloxacillin, amoxicillin, ampicillin, penicillin and oxacillin with an incidence varied from 77.8 to 100%.

The correlation between β lactamase producer isolates and antibiotic sensitivity test was illustrated in (Tables 10 and 11).

DISCUSSION

Coagulase-negative staphylococci (CNS) importance has increased and they have become the predominant pathogens isolated from subclinical mastitis in several countries **(Tenhagen *et al*., 2006; Lim *et al*., 2007).** In the present investigation, the prevalence of CNS among 804 milk samples collected from mastitic cows and buffaloes were studied. Identification of CNS pathogens has been based on conventional microbiological procedures which include growth on various media, observation of colony morphology and hemolysis patterns, Gram staining characteristics and using the API staph ID (BioMerieux) commercial kit. The obtained results regarding the main phenotypical characteristics are generally similar with those from the scientific literature **(Quinn *et al*., 2002; Buiuc and NeguŃ, 2008)**. It was clear that staphylococci specific primer (16srRNA) generated 228 bp amplicon from all CNS isolates. While the selected *S. aureus* primer (Nuc) generated 279 bp amplicon from *S. aureus* ATCC 25923 reference strain. The present data illustrated that, *S. aureus* was the predominant isolates recovered from the samples. The predominance of *S. aureus* among the mastitis causing agents was recorded earlier in cattle and sheep **(Ivana *et al*., 2010).** CNS was detected from SCM cows and buffaloes with incidence of 16.6 and 40.6 % respectively. The highest prevalence of inframammary infections with CNS was reported in Finland, where CNS were isolated from 50 % of the quarters positive for bacterial growth in a nationwide survey **(Pitkala *et al*., 2004).** In a similar survey in Norway, the prevalence of CNS was 16% **(Osteras *et al*., 2006)**. In Germany, CNS was isolated from 9% of the quarter milk samples in a total of 80 dairy herds, and they comprised 35% of samples positive for bacterial growth

(Tenhagen *et al*., 2006). In two dairy research herds in Ontario, Canada, CNS were the most common bacteria (51%) causing intramammary infection (IMI) at drying off **(Lim *et al*., 2007).** The proportion of CNS is generally high in samples collected from animals with subclinical mastitis but low in samples from animals with clinical mastitis. Reports on the clinical characteristics of CNS mastitis are scant as CNS has been ignored in many studies on clinical mastitis. In a recent Finnish study, half of the intramammary infections due to CNS were clinical, but in the majority of the cases the signs were very mild **(Taponen *et al*., 2006).** *S. lugdunensis, S. xylosus*, *S. cohnii*, *S. hominis*, *S. saprophyticus*, *S. lentus* and *S. simulans* were identified from the examined milk samples. The predominant species isolated from most herds are *S. chromogenes* and *S. hyicus*, but other staphylococci isolated commonly include *S. simulans*, *S. epidermidis*, *S. hominis, S. hemolyticus* and *S. xylosus* **(Sawanta *et al*., 2009, Thorberg *et al*., 2009).** *Staphylococcus equorum* was the predominant species, followed by *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* **(De Visscher *et al*., 2017). Piessens *et al*. (2011)** recorded that, the species causing IMI were *S. chromogenes*, *S. haemolyticus*, *S. simulans* and *S. epidermidis*, for *S. haemolyticus* and *S. simulans*, the environment was found as a reservoir, suggesting that IMI with these species were possibly environmental in origin. A huge variation in CNS species distribution among herds has been observed in several studies as recorded by **De Visscher *et al*. (2017).** It was concluded that CNS are emerging as important minor mastitis pathogens, can cause substantial economic losses, resulting in increased milk SCC which affects milk quality, and may be related to decreased milk production. Therefore, udder health is a critical factor, and control of intra-mammary infections is consequently of the greatest importance for dairy farmers. Among the examined CNS isolates it is clear that, 47.8% and 45.5% from cows and buffalo's isolates, respectively had creamy colonies, while 52.2% and 54.5%, respectively had white colonies. *S. aureus* isolated from cows and buffaloes had golden yellow colonies. Also 52.2% and 45.5% respectively were α hemolysis and 47.8% and 54.5%, respectively were β hemolysis on blood agar. Increasing antimicrobial resistance has become a serious concern worldwide and antimicrobial use in animal agriculture is currently under scrutiny. In the present study 45 CNS isolated from cow (23) and buffaloes (22), as well as 18 *S. aureus* (11from cow and 7 from buffaloes) were included in susceptibility analysis. The presence of β-lactamases in CNS has been observed both in human and veterinary isolates. **Taponen *et al.***

(2006) reported presence of β-lactamases in 19 % of CNS that caused mastitis in lactating dairy cows. Using β-lactams strips, β- lactamase activity was investigated among the CNS isolates as shown in the present data 22 (95.7 %) and 14 (63.6 %) CNS isolated from cows and buffaloes respectively were β- lactamase positive. Most of CNS isolates were sensitive to florfenicol, neomycin, gentamicin and erythromycin; meanwhile the examined isolates were resistant to cloxacillin, oxacillin, amoxicillin and ampicillin. **Sampimon *et al.* (2011)** concluded that CNS species isolated from bovine milk differ significantly in phenotypic and antimicrobial resistance profiles, 40.6% expressed resistance to a single compound or a single class of compounds, and 10.6% to multiple drug classes. CNS resistance to methicillin and other semi synthetic penicillins is now common **(Stuart *et al.*, 2011).** The presence of *mecA* has been detected in various species of staphylococci including *S. intermedius, S. epidermidis, S. lentus, S. saprophyticus, S. xylosus, S. sciuri,* and *S. haemolyticus* **(Mo and Wang, 1997; Gortel *et al.*, 1999; Yasuda *et al.*, 2000).** Methicillin-resistant *S. aureus* (MRSA) likely originated by acquisition of the staphylococcal cassette chromosome (SCC) from CNS **(Tulinski *et al.*, 2011).** It concluded that CNS strains are emerging as important minor mastitis pathogens and can be the cause of substantial economic losses. The present study revealed differences in antimicrobial susceptibility among the CNS species evaluated. Over 40 % of the tested staphylococci were resistant to at least one antimicrobial agent. The high resistance to penicillin plus the presence of Methicillin-resistant isolates found in this study emphasize the importance of identification of CNS among mastitic animals.

REFERENCES

American Public Health Association "APHA", (1992): Standard methods for examination of dairy products.INC. 16th ED.New York.

Blood DC, Handerson JA. (1986): Veterinary medicine.3rd edition, Baillier Tinfall and Gassel, London.

Buiuc D, NeguŃ M. (2008): Tratat de microbiologie clinică, ed. a II-a, Ed. Medicală, Bucuresti.

Cruickshank R, Duguid JP, Morromian BP., Swain RH. (1975): Medical microbiology 12 th Ed. Vol. 11, Churchil Living stone. Edinbergh, London and New York.

De Visscher A., Piepers S., Haesebrouck F., Supré K., De Vliegher S.(2017): Coagulase-negative Staphylococcus species in bulk milk: Prevalence, distribution, and associated subgroup- and species-specific risk factors 2017 journal of Dairy science.100 (1):629 - 642.

Finegold SM., Martin W.J. (1982): Bailey and Scott's Diagnostic Microbiology. 6th Ed., the C.V. Mosby Company, St. Louis, Torento, London.

Gortel K., Campbell KL., Kakoma I., Whittem T., and Schaeffer DJ., Weisiger R. M. (1999): Methicillin resistance among staphylococci isolated from dogs. Am. J. Vet. Res., 60:1526 - 1530.

Ivana P., Mangesh B., Eva D., and Milan T. (2010): Genotypic Characterization of Coagulase-negative Staphylococci Isolated from Sheep Milk in Slovakia. Acta. Vet. Brno. 79, 269-275.

Lim GH., Leslie KE., Kelton DF., Duffield TF., Timms LL., Dingwell RT., (2007): Adherence and efficacy of an external sealant to prevent new intramammary infections in the dry period.J. Dairy Sci. 90, 1289-1300.

Livermore DM, Brown DF. (2001): Detection of beta-Lactamase mediated resistance.J. Antmicrob Chemother. 2001 Jul; 48 Suppl 1: 59 - 64.

Mo L., Wang Q. (1997): Rapid detection of methicillin-resistant staphylo-cocci using polymerase chain reaction. Int J Infect Dis., 2:15-20.

Osteras O., Solverod L., Reksen O. (2006): Milk culture results in a large Norwegian survey effect of season, parity, days in milk, resistance, and clustering. J. Dairy Sci. 89, 1010 - 1023.

Pereira V., Lopes C., Castro A., Silva J., and Gibbs P., Teixeira P. (2009): Characterization for enterotoxin production, Virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various food in Portugal. Food Microbiol. 278-282.

Piessens V., Van Coillie E., Verbist B., Supré K., Braem G., Van Nuffel A., De Vuyst L., Heyndrickx M., De Vliegher S. (2011): Distribution of coagulase-negative *Staphylococcus* species from milk and environment of dairy cows differs between herds.J.dairy sci. 94, 2933-2944.

Pitkala A., Haveri M., Pyorala S., Myllys V., Honkanen-Buzalski T. (2004): Bovine mastitis in Finland 2001 - Prevalence, distribution of bacteria, and antimicrobial resistance.J. Dairy Sci. 87, 2433-2441.

Quinn PJ, Markey B., Carter ME., Donnely WJ, Leonard FC. (2002): Veterinary microbiology and microbial disease.Blackwell Science Ltd., TJ International Ltd, Padstow, Cornwall, Great Britain.

Radostits MO., Gay CC., Blood DC, Hinchcliff KW. (2000): Mastitis, In: Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, pigs, Goats and Horses. 9th Edition, W. B. Saunders Company Ltd.

Reischl U., Pulz M., Ehret W., Wolf H. (1994): PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol.Bio. Tech. 17, 844 - 845.

Ruegg PL. (2009): The quest for the perfect test: Phenotypic versus genotypic identification of coagulase-negative staphylococci associated with bovine mastitis. Vet. Microbiol. 134, 15-19.

Sampimon OC.,; Lam TJGM., Mevius DJ., Schukken, YH., Zadoks RN. (2011): Antimicrobial susceptibility of coagulase negative staphylococci isolated from bovine milk samples. Vet. Microbiol, 150: 173-179.

Sawanta AA., Gillespiea BE., Oliver SP. (2009): Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. Vet, Microbiol. 134, 73 - 81.

Schalm OW., Carroll EJ., Jain NC. (1971): Bovine Mastitis.Lea and Febiger, Philadelphia, PA.

Stuart JI., John MA., Milburn S., Diagre D., Wilson B., Hussain Z. (2011): Susceptibility patterns of coagulase-negative staphylococci to several newer antimicrobial agents in comparison with vancomycin and oxacillin. Int J Antimicrob Agents., 37: 248 -252.

Taponen S. (2008): Bovine mastitis caused by coagulase-negative staphylococci. Faculty of Vet. Medicine. University of Helsinki Finland.

Taponen S., Pyörälä S. (2009): Coagulase-negative Staphylococci as cause of bovine mastitis not so different from *Staphylococcus aureus*? Vet. Microbio, 134:29 -36.

Taponen S., Simojoki H., Haveri M., and Larsen HD., Pyorala S. (2006): Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Vet. Microbiol. 115, 199 - 207.

Tenhagen BA., Koster G., Wallmann J., Heuwieser W. (2006): Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci. 89, 2542-2551.

Thorberg BM., Danielsson-Tham ML., Emanuelson U. , Persson Waller K. (2009): Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci.

Tulinski P., Fluit AC., Wagenaar JA., Mevius D., van de Vijver L., Duim, B. (2011): Methicillin-resistant coagulase-negative staphylococci on pig farms act as a reservoir of heterogeneous SCCmec elements. Applied and Environmental Microbiology, 78 (2): 299 -304.

Yasuda R., Kawano J., Onada H., Takagi M., and Shimizu A., Anazi T. (2000): Methicillin-resistant coagulase-negative staphylococci isolated from healthy horses in Japan. Am. J. Vet. Res., 61: 1451 1455.

Zhang K., Sparling J., Chow BL., Elsayed S., Hussain Z., Church DL., Gregson DB., Louie T., Conly JM. (2004): New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J. Clin. Microbiol. 42, 4947 - 4955.

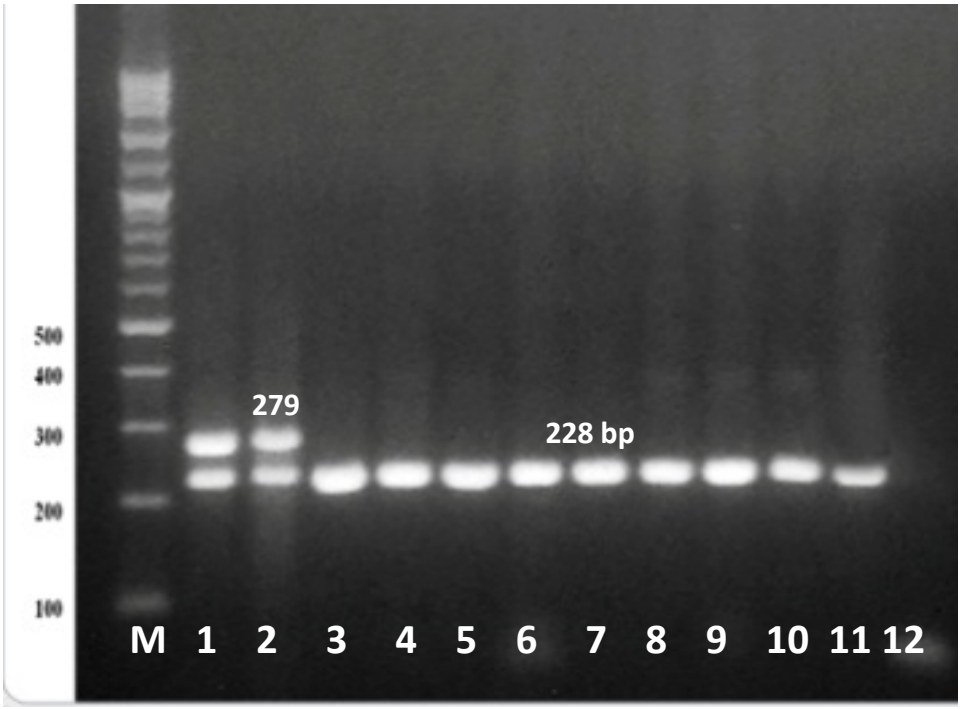

Fig. (1): the multiplex PCR assay to detect *Staphylococcus* isolates.

A): M: 100 bp DNA Ladder (Fermentas), Lanes 1and 2: *S. aureus* ATCC 25923 reference strain and *S. aureus* ATCC 43300 reference strain respectively showed 228 bp & 279 bp amplified bands, Lane 3 *S. epidermidis* ATCC 12228 reference strain showed 228 bp amplified band, Lanes: 4-11 CNS isolates had 228 bp only and Lane 12: control negative.

Table (1): The prevalence of staphylococci among the examined cows and buffaloes.

Isolated Bacteria	Cows						Buffaloes					
	State of milk sample						State of milk sample					
	Negative CMT (161)		Subclinical mastitic Samples SCM samples (459)		Clinical (12)		Negative CMT (40)		Subclinical mastitic Samples SCM samples (128)		Clinical (4)	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
S. aureus	66	41	235	51.2	12	100	6	15	47	36.7	4	100
CNS	35	21.7	64	13.9	-	0	8	20	52	40.6	-	0

N. = [Number of positive samples. % was calculated according to the total number of examined samples.

Table (2): Average of somatic cell count among the staphylococci infected cow and buffalo milk samples.

Isolated bacteria	Cow							Buffalo						
	Number of the examined samples	< 250,000		250,000-500,000		≥ 500,000		Number of the examined samples	< 250,000		250,000-500,000		≥ 500,000	
		N.	%	N.	%	N.	%		N.	%	N.	%	N.	%
S. aureus	313	67	21.4	49	15.7	197	62.9	57	13	22.8	23	40.4	21	36.8
CNS	99	38	38.4	34	34.3	27	27.3	60	22	36.7	26	43.3	12	20

N. = [Number of positive quarters.

% was calculated according to number of the examined samples.

Mean SCC up to 60,000 cell/ ml for CMS –ve samples.

Table (3): pigmentation activity among the CNS isolated from milk samples.

CNS	CNS Isolated From:									
	Cow					Buffalo				
	n	Creamy colonies		White colonies		n	Creamy colonies		White colonies	
		No	%	No	%		No	%	No	%
S. lugdunensis (n=7)	-	-	-	-	-	7	5	71.4	2	28.6
S. xylosus (n=14)	8	7	87.5	1	12.5	6	1	16.7	5	83.3
S. saprophyticus (n=4)	4	-	-	4	100	-	-	-	-	-
S. cohnii (n=6)	2	1	50	1	50	4	3	75	1	25
S. simulans (n=1)	-	-	-	-	-	1	-	-	1	100
S. lentus (n=1)	1	-	-	1	100	-	-	-	-	-
S. hominis (n=12)	8	3	37.5	5	62.5	4	1	25	3	75
Total (n=45)	23	11	47.8	12	52.2	22	10	45.5	12	54.5

Table (4): pigmentation activity among the *S. aureus* isolated from milk samples.

S. aureus	***S. aureus* Isolated From:**					
	Cow			**Buffalo**		
	n	**Golden Yellow colonies**		**n**	**Golden Yellow colonies**	
		No	**%**		**No**	**%**
***S. aureus* (n=18)**	**11**	**11**	**100**	**7**	**7**	**100**

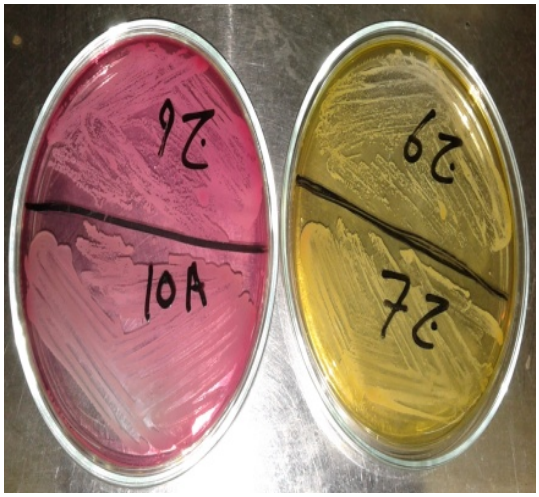

Fig (2): *S. aureus and* CNS on mannitol salt agar.

Table (5): Analysis of hemolytic activity among the CNS isolated from milk samples.

CNS	**CNS Strains Isolated From:**									
	Cow					**Buffalo**				
	n	**α**		**β**		**n**	**α**		**β**	
		No	**%**	**No**	**%**		**No**	**%**	**No**	**%**
***S. lugdunensis* (n=7)**	-	-	-	-	-	**7**	**2**	**28.6**	**5**	**71.4**
***S. xylosus* (n=14)**	**8**	**4**	**50**	**4**	**50**	**6**	**5**	**83.3**	**1**	**16.7**
***S. saprophyticus* (n=4)**	**4**	**3**	**75**	**1**	**25**	-	-	-	-	-
***S. cohnii* (n=6)**	**2**	**2**	**100**	-	-	**4**	**2**	**50**	**2**	**50**
***S. simulans* (n=1)**	-	-	-	-	-	**1**	-	-	**1**	**100**
***S. lentus* (n=1)**	**1**	**1**	**100**	-	-	-	-	-	-	-
***S. hominis* (n=12)**	**8**	**2**	**25**	**6**	**75**	**4**	**1**	**25**	**3**	**75**
Total (n=45)	**23**	**12**	**52.2**	**11**	**47.8**	**22**	**10**	**45.5**	**12**	**54.5**

Table (6): Analysis of hemolytic activity among the *S. aureus* isolated from milk samples.

S. aureus	***S. aureus* Strains Isolated From:**									
	Cow					**Buffalo**				
	n	**α**		**β**		**n**	**α**		**β**	
		No	**%**	**No**	**%**		**No**	**%**	**No**	**%**
***S. aureus* (n=18)**	**11**	**4**	**36.4**	**7**	**63.6**	**7**	**3**	**42.9**	**4**	**57.1**

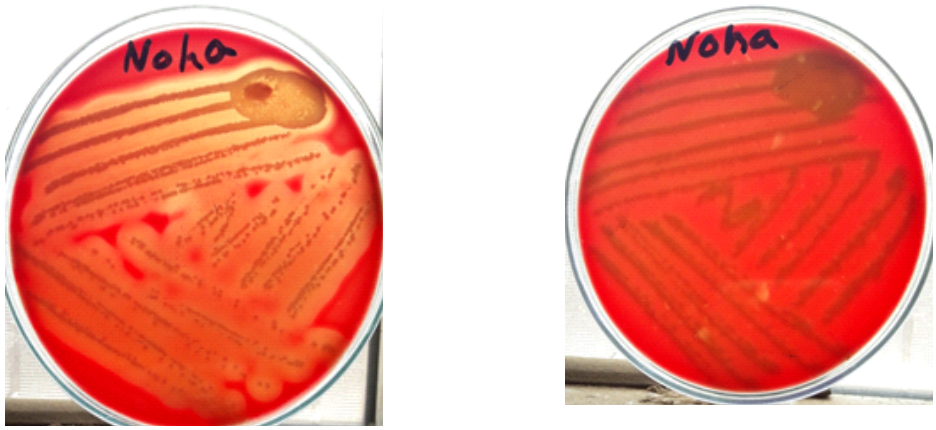

Fig. (3): CNS showed beta and alpha hemolysis on blood agar respectively.

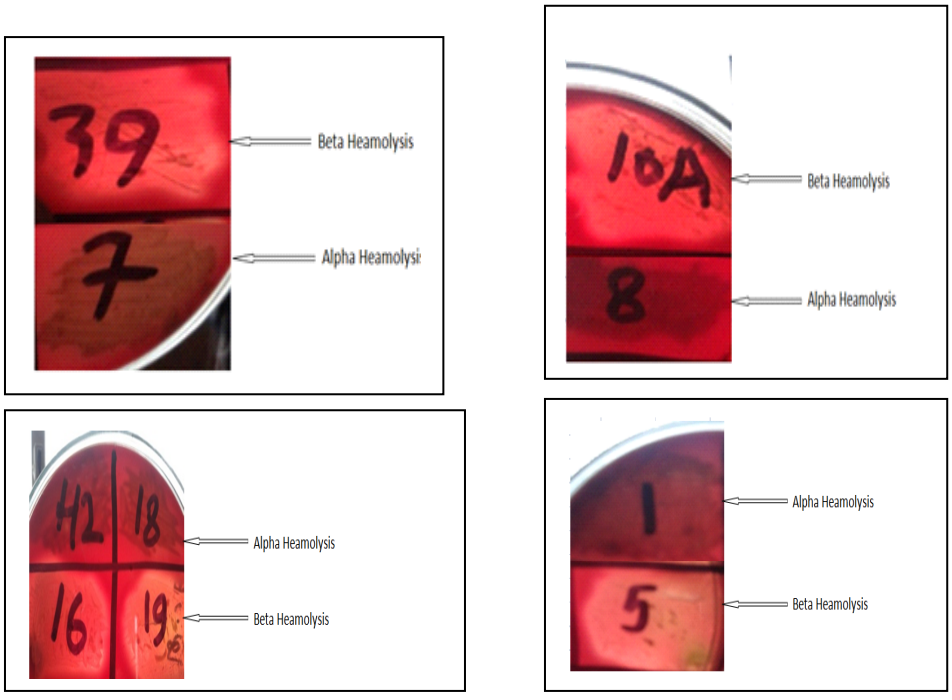

Fig.(4): CNS and *S. aureus* isolates showed alpha and beta hemolysis on blood agar.

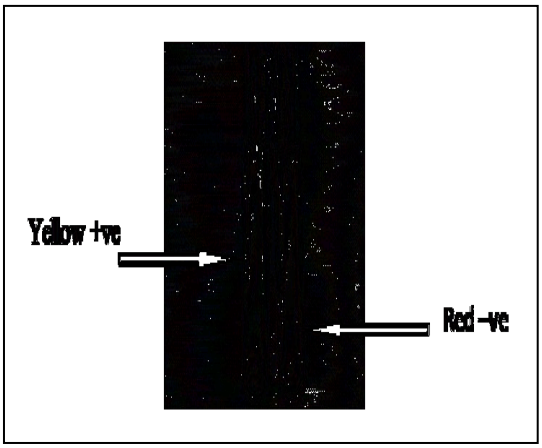

Fig. (5): β lactamase activity among CNS isolates.

Yellow +ve

Red –ve

Table (7): β- lactamase activity of the examined staphylococci isolates.

Isolates	Cow			Buffaloes		
	n	No.	%	n	No.	%
S. aureus (n=18)	11	9	81.8%	7	6	85.7%
CNS(n=45)	23	22	95.7	22	14	63.6

n= number of the examined isolates No. = Number of positive β-lactamase isolates.

Table (8): The antibacterial resistance among the *S. aureus* isolated from.

Antibiotics	*S. aureus* strains isolated from:					
	Cow milk (n=11)		Buffalo milk (n=7)		Total (n=18)	
	No.	%	No.	%	No.	%
AMC	7	63.6	5	71.4	12	66.7
AMP	10	90.9	7	100	17	94.4
AML	10	90.9	7	100	17	94.4
OT	4	36.4	4	57.1	8	44.4
P	11	100	7	100	18	100
CX	10	90.9	6	85.7	16	88.9
FFC	6	54.5	4	57.1	10	55.6
N	5	45.5	3	42.9	8	44.4
G	7	63.6	5	71.4	12	66.7
O	10	90.9	7	100	17	94.4
E	4	36.4	3	42.9	7	38.9

N: number of examined *S.aureus* isolates, No.:number of resistant isolates, % was calculated according to the number of examined *S. aureus* isolates, AML: Amoxicillin (10 µg/disk), AMC: amoxicillin + clavulanic acid (20+10 µg/disk), AMP: Ampicillin (10 µg/disk), CX: Cloxacillin (5 µg/disk), E: erythromycin (15 µg/disk), FFC: Florfenicol (30 µg/disk), G: Gentamicin (10 µg/disk), N: Neomycin (30 µg/disk), O: Oxacillin (1 µg/disk), OT: oxytetracycline (30 µg/disk) and P: Penicillin G (10units/disk).

Table (9): The antibacterial resistance among the CNS (n=45) isolated from milk samples.

Antibiotics	Total	
	No.	%
AMC	24	53.3
AMP	41	91.1
AML	45	100
OT	13	28.9
P	38	84.4
CX	45	100
FFC	2	4.4
N	0	0
G	2	4.4
O	35	77.8
E	6	13.3

N: number of examined *S. aureus* isolates, No.: number of resistant isolates,% was calculated according to the number of examined *S. aureus* isolates, AML: amoxicillin (10 µg/disk), AMC: amoxicillin + clavulanic acid (20+10 µg/disk), AMP: ampicillin (10 µg/disk), CX: cloxacillin (5 µg/disk), E: erythromycin (15 µg/disk), FFC: florfenicol (30 µg/disk), G: gentamicin (10 µg/disk), N: neomycin (30 µg/disk), O: oxacillin (1 µg/disk), OT: oxytetracycline (30 µg/disk) and P: penicillin G (10units/disk).

Table (10): Correlation between β- lactamase *S. aureus* producers (n=15) and resistant to antibacterial agents among cow and buffalo isolates.

Antibiotics	No. of examined isolates	
	No.	%
AMC	12	80.0
AMP	15	100
AML	15	100
OT	7	46.7
P	15	100
CX	15	100
FFC	8	53.3
N	6	40
G	10	66.7
O	15	100
E	5	33.3

N: number of examined *S. aureus* isolates, No.: number of resistant isolates, % was calculated according to the number of examined *S. aureus* isolates, AML: amoxicillin (10 µg/disk), AMC: amoxicillin + clavulanic acid (20+10 µg/disk), AMP: ampicillin (10 µg/disk), CX: Cloxacillin (5 µg/disk), E: erythromycin (15 µg/disk), FFC: florfenicol (30 µg/disk), G: gentamicin (10 µg/disk), N: neomycin (30 µg/disk), O: oxacillin (1 µg/disk), OT: oxytetracycline (30 µg/disk) and P: penicillin G (10units/disk).

Table (11): Correlation between β- lactamase CNS producers and resistant to antibacterial agents among cow isolates.

CNS	AMC		AMP		AML		OT		P		CX		FFC		N		G		O		E	
	N.	%	N.	%	N.	%	N	%	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
cow isolates (n=22)	12	54.5	22	100	22	100	2	9.1	16	72.7	22	100	0	0	0	0	0	0	19	86.4	4*	18.2
buffalo isolates (n=14)	6	42.8	10	71.4	14	100	8	57.1	14	100	14	100	2	14.3	0	0	2	14.3	10	71.4	0	0
Total (n=36)	18	50	32	88.9	36	100	10	27.8	30	83.3	36	100	2	5.6	0	0	2	5.6	29	80.6	4	11.1

N: number of examined *S. aureus* isolates, No.: number of resistant isolates, % was calculated according to the number of examined *S. aureus* isolates, AML: amoxicillin (10 µg/disk), AMC: amoxicillin + clavulanic acid (20+10 µg/disk), AMP: ampicillin (10 µg/disk), CX: cloxacillin (5 µg/disk), E: erythromycin (15 µg/disk), FFC: florfenicol (30 µg/disk), G: gentamicin (10 µg/disk), N: neomycin (30 µg/disk), O: oxacillin (1 µg/disk), OT: oxytetracycline (30 µg/disk) and P: penicillin G (10units/disk).