Impacts of subclinical mastitis on milk quality, clotting ability and microbial resistance of the causative Staphylococci

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SUMMARY

Bovine mastitis is one of the most problematic diseases and continues to be a leading cause of heavy economic losses in the dairy industry and a public health hazard globally. To understand the characteristics of subclinical mastitis (SCM) in lactating cows and their associated effects on milk quality, protein composition, and milk clotting ability, 240 quarter-milk samples were collected and tested by California Mastitis Test (CMT). Milk composition was analyzed using LactoScope FT-A and separation of protein fractions was performed by PAGE-SDS electrophoresis. We also measured the time from rennet addition to milk gelation (RCT) as a traditional milk coagulation trait. Samples with SCM were analyzed bacteriologically, and Staphylococci isolates were tested for antibiotic susceptibility. Higher values of conductivity and pH were recorded from CMT-positive milk samples. Overall, 50/240 (20.83%) quarters suffered from SCM, whose 64% (32/50) infected with Staphylococci. On the 36 tested Staphylococci, resistance to penicillin and erythromycin represented 83.3%, and 61.1% respectively. Resistance to cefoxitin was linked to three isolates while 77.7% were multi-drug resistant, but in proportion that differ between *S. aureus* (88.8%) and non-aureus Staphylococci (74.1%). Physico-chemical analysis indicated that, quarters with SCM had lower milk-fat content and mineral content compared with quarters without SCM. The profiles of total proteins electrophoresis revealed degradation of casein fractions in milk with SCM. Milk samples subclinically infected with Staphylococci exhibited longer coagulation time (1093.9±781.9 seconds) and weaker clotting activity (2.55±1.49 RU) than milk samples collected from healthy quarters which showed 325.3±177.5 seconds and 7.80 ± 4.46 RU. The increase in conductivity due to intramammary infection, was highly associated with an elongation in RCT. Moreover, clotting activity was inversely proportional to conductivity. Due to its impacts on milk composition, proteins integrity and clotting ability, SCM still a major concern in dairy industry which needs efficient measures to control their occurrence in dairy herds.

KEY WORDS

Antibiotic susceptibility, clotting ability, milk composition, Staphylococci, subclinical mastitis.

INTRODUCTION

Achievement of high yield and quality products in the dairy processing industry depends on the quality of raw milk (1). The latter is still affected by several environmental and individual factors, including cow's health status (2). Indeed, nutritional values and content of milk which are important for human nutrition, may be depreciated with a systemic or mammary gland infection of host animals. Bovine mastitis is a complex disease, mainly caused by a variety of pathogens, with substantial differences in infection patterns with no simple model encompassing all possible facets of the disease (3). Antibiotic therapy is commonly implemented for prevention and control of mastitis; unfortunately, despite the best possible antimicrobial treatments available, bacteriological cure failure is common, especially of intramammary infections (IMI) associated with *S. aureus* (4). This situation could lead to the persistence and transmission of multidrug-resistant bacteria in dairy farms. Mastitis represents one of the most economically important health traits for milk production which makes it among the major concerns for the livestock sector (5). In cases of subclinical mastitis (SCM), no visible abnormalities of the milk or udder can be observed, and tests are needed to detect the inflammatory responses following IMI. For their detection, methods such as the California mastitis test (CMT), Somatic Cells Count (SCC), certain biochemical methods, bacteriological examination of milk and electrical conductivity have been suggested (6). SCM usually leads the clinical form as it is of longer period, difficult to diagnose, adversely affects milk production and quality and comprises a reservoir of pathogens affecting other animals within the herd (3,7). Increased SCC (higher than

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200000cells/mL) in milk is commonly used as indicator of SCM and reflects the onset of an immune response to the presence of IMI. High SCC in milk reduces the quality of milk and dairy products, affects shelf life and flavor of milk, and deteriorates the physicochemical properties and cheese-making traits of milk (2,8,9). Besides, a very low SCC (lower than 150000cells/mL) was also reported to have a negative effect on some milk technological traits, which could be associated with an ineffective response to an undetectable mastitis event (2). Therefore, the objectives of this current study were to characterize SCM in lactating cows and their relationship with milk quality, protein composition, and milk clotting ability.

MATERIAL AND METHODS

Study area and cows

Animals were selected from eight dairy herds located in the department of Tizi-Ouzou (Algeria). Sixty lactating cows were enrolled in the present study. The average herd size was 7.5 ranging from 5 to 17 cows /herd. Breeding management followed an extensive and sometimes intensive mode. Cows included in this study were selected randomly within herds' accessibility of the breeders. Age, lactation stage and parity were not considered in the choice of animals.

Screening for mastitis

After obtaining permission from farms owners, udders of the cows were first examined by visual inspection and palpated for the presence of any lesion, pain, heat and swelling. Any abnormality in color or consistency of milk collected from each quarter was checked. CMT was performed on the clinically healthy udder-quarters at the post-colostral period to determine the presence of SCM (10). Milk samples from 240 udder-quarters were collected from the 60 cows and tested using CMT. When a quarter showed no visible signs of clinical mastitis but revealed positive to CMT, it is considered impaired with SCM.

Samples collection from cows

Two different milk samples were collected before the morning milking according to National Mastitis Council (NMC) guidelines (11). For bacteriological analysis, teat ends were cleaned externally with commercial pre-milking disinfectants, dried with individual towels and cleaned again with alcohol. After discarding the first streams of foremilk, approximately 10mL of milk from each quarter was collected in sterile tubes. Immediately after aseptic collection of milk samples, approximately 100mL of milk was manually collected from each CMT-positive and CMT-negative quarters. The latter sample was dedicated for the analysis of milk composition and cheese-making traits. All quarter-milk samples individually collected were stored at 4°C, and submitted to the laboratory within 2-4 hours.

Bacteriological analysis

Bacteriological analysis was performed exclusively on CMT-positive quarter-milk samples according to NMC standards (11). Briefly from each sample, 0.01mL of milk was plated both on mannitol salt agar medium and blood agar. Cultures were examined after being incubated aerobically for 24 h or 48 h at 37°C. A mammary quarter was considered culture positive when the growth of at least one colony was detected on the streaks. Samples yielding more than two different bacterial species were considered to be contaminated. Bacteria were identified based on colony morphology and biochemical tests. Catalase tests with hydrogen peroxide 3% were used to differentiate between catalase positive Staphylococci and catalase negative cocci. Coagulase tests were carried out using sterile rabbit plasma to distinguish *S. aureus* from non-aureus Staphylococci.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out with equivalence of 0.5 McFarland turbidity standards by agar disc diffusion method on Mueller-Hinton agar plates following the guidelines of Clinical and Laboratory Standards Institute (12). The reference strain (*S. aureus* ATCC 25923) was used as a control for the disc diffusion technique. The isolated strains were tested for their susceptibility to cefoxitin (indicative for methicillin resistant Staphylococci (MRS)), oxacillin, penicillin, tetracycline, spiramycin, erythromycin, clindamycin, vancomycin, fusidic acid and chloramphenicol.

Milk composition analysis and proteins separation

All analytical evaluations have been completed in duplicate for each quarter-milk sample. Milk was tested within 24h of collection for fat, solids non-fat, protein, lactose (%), and mineral substances using a LactoScope FT-A Results Plus (PerkinElmer, Inc; Connecticut, U.S.A). This tool also recorded electrical conductivity (EC), density, and pH. This latter was compared with the milk pH recorded at room sample temperature using a pH-meter (HANNA Instruments, Lingolsheim, France). Milk protein fractions were separated in the presence of Sodium Dodecyl Sulfate (SDS 10%, w/v) and 2-mercaptoethanol (4%, v/v) by PAGE-SDS electrophoresis.

Milk clotting aptitude determination

We measured under standardized conditions, the rennet coagulation time (RCT) as a traditional milk coagulation trait (13). Coagulation was observed on a thin sheet of milk in a rotating tube set on a black background. By reproducing the clotting protocol performed in small-scall industries, we have prepared a reference sample to which we have compared the results of the samples tested. The method is to add 1µL of enzyme solution to 2mL of standard substrate (reconstituted milk powder) at 38°C and then record the coagulation time. The procedure was repeated twice in order to achieve more reliable results.

The preparation of the enzymatic solution consists in dissolving 22mg of the coagulating enzyme: recombinant bovine chymosin in 5mL of distilled water, followed by magnetic stirring. The preparation of the standard substrate consists of dissolving milk powder of the low heat at 0% (w/v) in a solution of $CaCl_2$ (0.01 M) and adjusting the pH to 6.5 by adding a solution of NaOH (0.1). The standard substrate is then divided into 2 test tubes (2mL/tube). The addition of the coagulant extract was performed within 10µL/2mL of the standard substrate. Immediate and rapid homogenization is done. In the Bain Marie, the three successive reversals of the mixture after 30 seconds correspond to time zero. The selected healthy and mastitis milk samples were also distributed in test tubes within 2mL each and 10µL of the enzyme solution were added respectively. Before being placed in the Bain Marie 38°C, a quick and immediate homogenization was achieved. RCT was recorded by a stop-

watch and clotting activity was calculated with the following formulas:

Clotting Activity =10 x Standard Milk Volume / Enzyme Volume x RCT
Clotting Activity: Rennet Unit (RU)
Standard Milk Volume: 2 mL
Enzyme Volume: 0.01 mL
RCT: Rennet addition to milk gelation (seconds)

Statistical analysis
Raw data were entered to Microsoft Excel for Windows (2010; Microsoft Corp., Redmond, WA, USA) and imported to SPSS software version 20.0 (IBM Corp., Armonk, NY, USA) for statistical analysis. Initial descriptive statistics were done to summarize data while comparisons between averages were performed using Student test. Pearson's correlation analysis was used to establish the relationship between conductivity and coagulation characteristics in milk. A p-value of 0.05 was used to determine the significance level.

RESULTS

Prevalence of subclinical mastitis
Mastitis in its clinical and subclinical forms was diagnosed in 28 lactating cows. While moderate clinical (one quarter) and subclinical (three quarters) mastitis co-existed in one cow, one quarter was affected with moderate clinical form in another cow. Clinical mastitis wasn't be considered for further analysis.

From SCM prevalence stand point, all herds experienced SCM with 45% (27 of 60) affected cows and 20.83% (50 of 240) affected quarters. Most of the cows with SCM (13 of 27; 48.1%) had only one affected quarter, whereas 29.6 % (8 of 27) of the cows were diagnosed with SCM in two quarters, 11.1% (3 of 27) had three quarters affected, and 11.1% (3 of 27) had SCM in all four quarters (**Table 1**).

Bacterial analysis and intramammary infection
Only CMT-positive quarter-milk samples were analyzed bacteriologically. At least one bacterial species was isolated from 92% (46/50) of the cultured CMT-positive milk samples. Fifty-two isolates (one to two bacterial strains recovered after culture of CMT-positive samples on blood agar) were recovered from 46 positive milk samples. Samples showing two mixed bacteria species represented 12% (6 of 50). The most commonly isolated udder pathogen was *Staphylococcus* within 64% (32 of 50) of the CMT-positive milk samples, giving a quarter prevalence of 13.33% (32 of 240). The frequencies of Non-aureus Staphylococci (NAS) and *S. aureus* isolation in milk samples represented 50% and 18% respectively (**Table 2**).

Antibiotic susceptibility of Staphylococci
Antibiotic resistance determination revealed that only 8.3% of the Staphylococcal isolates (3/36) were susceptible to all the tested antibiotics. Higher levels of resistance were associated with penicillin, and erythromycin with 83.3% and 61.1% respectively. Resistance to cefoxitin was observed from three isolates.
Multi-drug resistance was observed in 77.7% of the tested isolates, but in proportion that differ between *S. aureus* (88.8%)

Table 1 - Screening for subclinical mastitis and associated milk characteristics.

Mastitis screening	Total	SCM/CM	Healthy	P-value
Farms	8	8 (100%) / 1 (12.5%)	0	/
Cows	60	27 (45%) / 2 (3.32%) *	32 (53.33%)	/
Quarters	240	50 (20.83%) / 2 (0.41%)	188 (78.33%)	/
Analyzed milk samples				
Bacteriological analysis of CMT-positives	50	50 (100%)	0	/
Physico-chemical analysis	74	49 (66.21%)	25 (33.78%)	/
Milk clotting aptitude analysis	31	17 (54.83%)	14 (45.16%)	/
Physico-chemical analysis	**Mean±sd**	**Mean±sd**	**Mean±sd**	**P-Value**
Milk features				
pH	6.53±0.24	6.56±0.23[a]	6.44±0.22[b]	P<0.001
Conductivity	5.27±0.03	5.47±0.62[c]	4.93±0.38[d]	P<0.001
Density	33.12±4.67	33.15±5.49	32.90±3.07	P=0.75
Milk components (g/100g)				
Fat	3.65±0.58	3.58±0.64	3.74± 0.43	P=0.08
Total proteins	3.05±0.48	3.06±0.55	3.01 ±0.36	P=0.52
Lactose	4.61±0.69	4.61±1.5	4.59± 0.43	P=0.9
Mineral substances	0.70±0.23	0.65±0.24[a]	0.77±0.19[b]	P=0.001
Solids non-fat	8.33±1.19	8.36±0.14[b]	8.24± 0.79[c]	P<0.001

sd: Standard deviation; SCM: Subclinical mastitis: CM: Clinical mastitis;
NB. *: One cow showed 3 quarters with SCM and one quarter with CM

Table 2 - Prevalence and bacterial isolation frequencies from bovine mastitic milk samples.

Analysis	No. of samples	Frequency %	Prevalence %	No. of isolates
Quarter milk screening	240	—	—	—
Samples positive to CMT	50	100	20.83	—
Negative cultures for CMT-positive samples	4	8	1.66	0
Positive cultures for CMT-positive samples	46	92	19.16	52
Positive cultures with one type of colonies	40	80	16.66	40
Positive cultures with two types of colonies	6	12	2,5	12
Cultures with an undetermined bacterium	16	32	6.66	16
Cultures with *S. aureus*	9	18	3.75	9
Cultures with NAS (Non-aureus Staphylococci)	25**	50	10.41	27

**: Cultures with two distinct colonies for NAS; Isolates: bacterial isolates

and NAS (74.1%). Of the twenty-four characterized patterns of resistance, a high diversity of profiles was detected among the NAS with 17 patterns than *S. aureus* isolates with seven different patterns (**Table 3**).

Physicochemical characteristics

Milk composition results were obtained from 74 quarter-milk samples. The overall means were as follows: 4.61% (± 0.69) lactose, 3.05% (± 0.48) protein, 3.65% (± 0.58) fat, 8.33% (± 1.19) nonfat solids, and 11.98% (±1.77) total solids contents in all samples. CMT-positive milk samples showed higher values of conductivity ($p<0.001$) and pH ($p<0.001$). Moreover, SCM increased solids non-fat contents (SNFC: $p<0.001$) but reduced mineral substances ($p=0.001$). No difference was found in milk fat content (MFC: $p=0.08$), total proteins ($p=0.52$) and lactose ($p=0.9$) between infected and uninfected quarters (**Table 1**). In opposite to milk samples from healthy quarters, milk samples with SCM showed well focused and more intense Igs, lactoferrins and BSA bands and less intense and non-focused bands for α-lactalbumin and β-lactoglobulin. The behavior of caseins fractions varied from one mastitic-quarter to another depending on the severity of the infection. Indeed, degradation of caseins in milk with SCM was mostly high, especially for κ-CN>> β-CN > α S2-CN. This latter was characterized by unfocused bands in form of patches losing their properties of migration (**Figure 1**).

Milk clotting ability

Thirty-one milk samples were subjected for milk coagulation trait analysis. The mean RCT recorded from mastitis quarter milk samples (1093.9±781.9 seconds) was higher ($p<0.001$) than that of the healthy (325.3±177.5 seconds) milk samples. Furthermore, the clotting activity was lower (2.55±1.49 RU) from mastitis milk samples than the healthy milk samples (7.80±4.46 RU). The clotting activity recorded upon the low heat 0% MFC control was higher from that recorded from mastitis and healthy milk. The increase in conductivity due to IMI, was highly associated with an elongation in RCT ($R=0.69$: $p<0.001$). Moreover, clotting activity was inversely proportional to conductivity ($R=-0.38$: $p=0.03$) (**Table 4**).

DISCUSSION

Udder health

At least one mastitis case was diagnosed from the entirely investigated herds in the present study, the detected prevalence of SCM at the quarter level was 20.83%. This relatively high

Table 3 - Antibiotic resistance patterns of Staphylococci involved in bovine subclinical mastitis.

Drug	Staphylococci		*S. aureus*		NAS	
Resistance	N	Rate	No.	Patterns of resistance	No.	Patterns of resistance
Total	36	—	9	No.=7	27	No.=17
6 antibiotics	2	5.5	1	{P,FX,E,VA,FA,CD}	1	{P,OX,FX,E,FA,CD}
5 antibiotics	3	8.3	0	-	3	{P,OX,TE,E,CD}, {P,TE,E,FA,CD}, {P,SP,TE,E,CD}
4 antibiotics	5	13.8	0	—	5	{P,E,FA,CD}, {P,TE,E,CD}, {P,C,E,CD}
3 antibiotics	11	30.5	3	{P,TE,E}, {P,C,CD}	8	{P,FX,OX}, {P,TE,E}, {P,E,CD}, {P,TE,FA}, {P,E,FA}, {TE,E,CD}
2 antibiotics	7	19.4	4	{P,E}, {P,C},{P,SP}, {P,TE}	3	{P,E}
1 antibiotic	5	13.8	0	—	5	{P}, {E}, {C}
SENSBLE	3	8.3	1	—	2	—

P. Penicillin; **OX.** Oxacillin; **FX.** Cefoxitin; **E.** Erythromycin; **TE.** Tetracyclin; **CD.** Clindamycin; **FA.** Fusidic Acid; **VA.** Vancomycin; **SP.** Spiramycin; **C.** Chloramphencol.

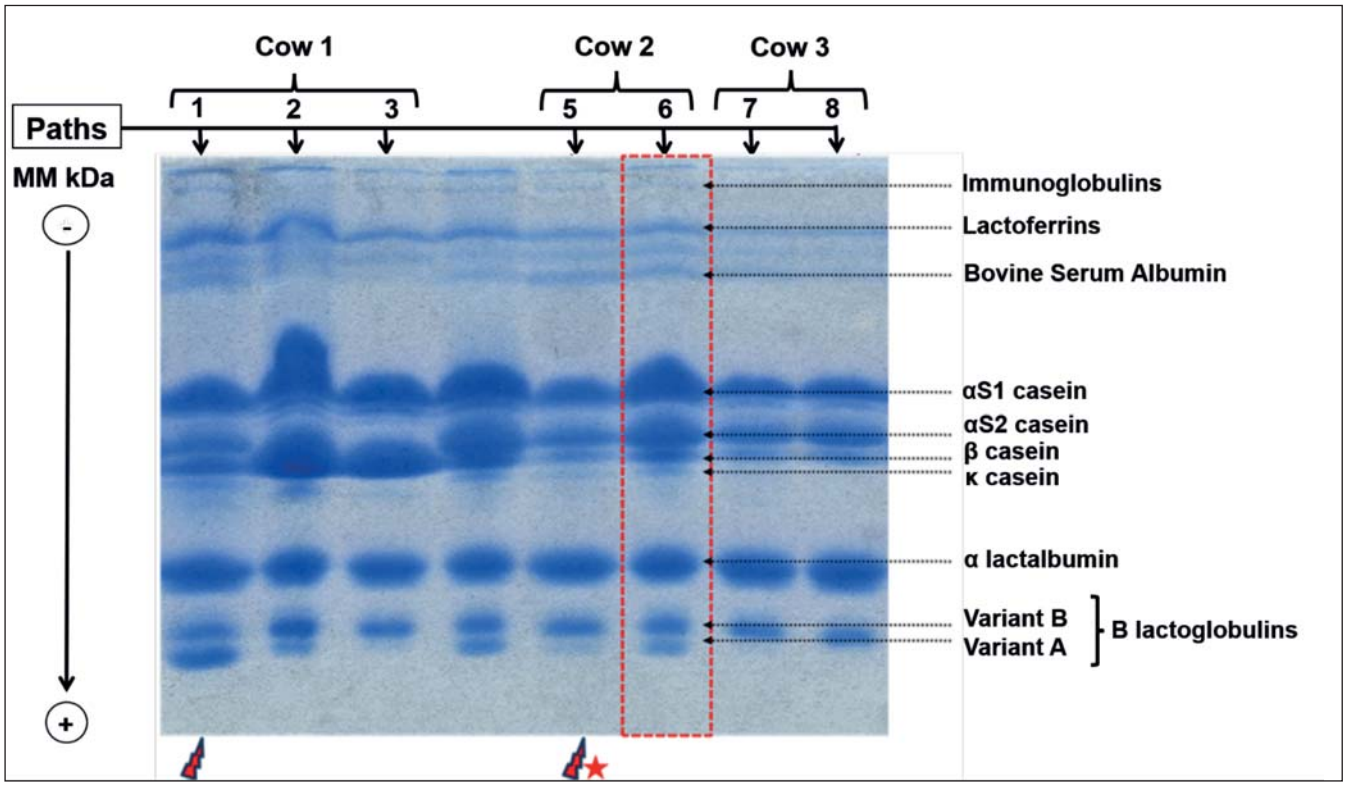

Figure 1 - Electrophoretic profiles of total proteins in PAGE-SDS.
CMT-Positive : 1(RAQ) ; 5 (RPQ) ; 7 (RPQ)
CMT-Negative: 2 (LAQ); 3 (RPQ); 6 (LPQ); 8 (LPQ)
RAQ: Right Anterior Quarter; **RPQ**: Right Posterior Quarter; **LAQ**: Left Anterior Quarter; **LPQ**: Left Posterior Quarter; **MM**: Molecular Mass

prevalence of bovine SCM was similar to the results obtained in other previous Algerian studies (14). The increased incidence of SCM in dairy livestock could be due to a lack of implementation of regular mastitis prevention and/or control strategies other than treating clinical cases. Although CMT is still used in most of studies due to its convenience and had been validated in field applications for SCM detection (6), none of the farmers were doing CMT or other tests routinely to screen their cows for SCM. Milk conductivity is considered an indicator of udder health as a result of changes in the ion balance associated with the inflammatory response to IMI (15). We observed an increase in milk conductivity and pH in quarters positive to CMT.

Contagious mastitis is considered of fairly vital significance to the public health as it is linked with many zoonotic diseases in which milk turns as a vehicle for the infectious agents (16,17). This highlights the importance of hygiene and managemental practices inside dairy farms. Moreover, it would be a serious hazard for public health because that mastitic milk is usually added into a bulk milk tank, especially in populations where some people could consume raw milk or non-heat-treated dairy products like yogurt or cheese (18). According to the microbiological finding of the study, Staphylococci were blamed from 64% of mastitic-quarters accounting 18% of *S. aureus* and 50 % of NAS. Consequently, co-infection with Staphylococci was detected in two quarters. *S. aureus* is considered as the main contagious mastitis causative agent with high ability to persist inside the udders. Its pathogenicity is based on the presence of important mechanisms such us its ability to form biofilms, polysaccharide capsule small colony variants, and their ability to invade professional and nonprofessional cells, which will protect *S. aureus* persistence from the innate and the adaptive immune response of the cows, and from antibiotics (19). The preponderance of NAS species in the study animals has also been observed in many other studies. Indeed, NAS have become the most common bovine mastitis isolate in many countries and could therefore be described as emerging mastitis pathogens (20). The dominance of this group of pathogens is possibly as a result of poor milking hygiene. NAS commonly colonize the teat end and teat canal only and are difficult to associate with clinical mastitis; under some circumstances however, they may lead to raised somatic cell counts and subclinical mastitis (20). Since they are a contagious and common colonizer of the teat-end and teat canal, the use of dry cow therapy and post-milking teat disinfectants are of great value in controlling the disease. These control measures however, were not used by most of farmers that participated in the study.

Antibiotic resistance has increased among various bacterial pathogens which is considered an emerging problem with a major public health concern due to the risk of resistance transmission to human as well as its influence on the effectiveness of the current antibiotic therapy (17,21). From bovine mastitis standpoint, the failure in the treatment always occurred due to: chronic infection accompanied with fibrosis, inadequate dose of antibiotics, and emergence of multidrug-resistant bacterial pathogens (22). We reported herein, high rates of antimicrobial resistance among Staphylococci stains involved in bovine mastitis, especially for penicillin and erythromycin with 83.3%, and 61.1% respectively. Similar levels of resistance have been reported previously, to the first-line treatment with penicillin in Staphylococci strains associated with bovine mastitis in Algeria (23). Additionally, cefoxitin resistance was used to determine the methicillin-resistant Staphylococcus isolates, and antimicrobial susceptibility testing showed three strains were

Table 4 - Clotting ability comparison between mastitic and non-mastitic milk samples.

/	Code	Rennet Clotting Time					Mean	CA (RU)
Standard control	T_1	852					865	2.31
	T_2	878						
S. aureus	Positive control	Conductivity	RCT(s)	CA (RU)	Negative control	Conductivity	RCT(s)	CA (RU)
	$V_4.PD^+$	5.41	1618	1.23	V.AG$^-$	5.09	146	13.69
	$KV_5.PG^+$	6.50	3536	0.56	$KV_4.AG^-$	5.51	511	3.91
	$O_1.PD^+$	4.9	425	4.70	$O_2.PD^-$	5.27	365	4.87
	$O'_1.PG^+$	4.34	426	4.16	$O'_1.PD^-$	4.35	277	7.22
	$O'_3.AG^+A$	4.60	336	5.95	$O'_3.AD^-$	4.50	252	7.93
	$O'_4.AG^+A$	4.97	1143	1.74	$O'_4.PD^-$	4.75	264	7.57
	8. AG^+	5.13	795	2.51	9. AD^-	5.17	693	2.88
	$T_1.PG^+$	5.43	1434	1.39	$T_2.PD^-$	4.93	278	7.19
NAS	$O_6.PD^+$	5.65	1245	1.60	$O_7.PD^-$	4.27	115	17.39
	$V_1.PD^+$	5.39	930	2.15	$V_1.PG^-$	4.75	172	7.35
	$KV_1.AG^+$	4.44	656	3.04	$KV_1.PD^-$	5.46	303	6.60
	6. AG^+	4.86	551	3.62	6. PD^-	5.65	134	14.92
	$V_2.AD^+$	6.79	1898	1.05	$V_2.AG^-$	4.61	594	3.35
	$O_4.AD^+$	5.65	637	2.27	$O_4.PG^-$	5.1	450	4.44
	$O'_4.PG^+$	4.77	1105	1.80	-	-	-	-
	$O_2.PG^+$	5.31	470	4.25	-	-	-	-
	$KV_1.AD^+$	5.22	1392	1.43	-	-	-	-
	Mean	**5.25**	1093.9	**2.55**	**Mean**	**4.95**	325.3	**7.80**
	Sd	**0.65**	781.9	**1.49**	**Sd**	**0.43**	177.5	**4.46**

T1: Standard control low heat: MFC:0%.

resistant to cefoxitin. For technical considerations, PCR have not been used for detection of *mec*A and/or *mec*C genes in the present study. The tested isolates showed 77.7% of multi-drug resistance, but in proportion that differ between *S. aureus* (88.8%) and NAS (74.1%). The relatively high resistance spectrum of Staphylococci involved in bovine mastitis is likely due to frequent and long-term use of antibiotics in therapeutics.

Milk quality and transformation ability

Variations in milk composition due to mastitis may impair the transformation process and the quality of dairy products (8). Our data from naturally occurring SCM cases in lactating cows, indicated that IMI affects negatively quarter milk composition. It has been shown that the degree of changes depends on the inflammatory response, bacterial pathogenicity as well as the severity and amount of affected tissue in the mammary gland (24). In cases of SCM, increased plasmin activity results impaired functional and secretory capacity of the mammary gland's epithelial cells (8,25), leading to a decreased MFC in milk (26). However, despite the well-established negative effect of plasmin on mammary epithelial cells' synthetic and secretory activity during IMI, literature results on the effect of SCM on MFC are contradictory. The latter supports the findings of the present study where impaired quarters by SCM produced milk with non-significant decreased MFC. Hence, the MFC increases due to the decreased milk volume in the infected glands (27).

Our protein content analysis was based mainly on the total protein value. It has been observed that milk samples from infected quarters have higher total protein and whey protein values but lower casein content when compared to milk samples from healthy quarters (1). The percentages of casein and whey protein were not calculated in the present study. Indeed, the disruption of the mammary gland's epithelium increased permeability of the milk barrier and facilitates the passage of serum proteins into the milk (25). Meanwhile, proteinases originating from bacteria and leucocytes in the mastitic milk cause the proteolysis of caseins, leading to a decrease in the casein content of milk (25,28). It seems herein that the decrease in the casein content was enough to compensate for the increase in the milk whey protein content which finally results in non-significant effect of SCM on total protein content in milk.

The levels of fat and protein contents evidenced good nutritional and cheese making quality of milk. Thereby, the coagulation process of milk starts with hydrolysis of κ-CN by the chymosin of rennet followed by the aggregation of casein micelles which form a reticulum entrapping the soluble phase and fat globules (29). The number of secondary interactions within the curd increases over time leading to its syneresis and partial expulsion of whey. In the present study, milk samples subclinically infected exhibited longer coagulation time and weaker clotting activity than milk samples collected from healthy

quarters. Deteriorating of coagulation properties could be attributed mainly to the higher milk pH and the degradation of casein fractions. Indeed, greater casein breakdown has a relevant effect on the technological behavior of bovine milk; while higher milk pH causes a decrease in the enzyme activity involved in milk clotting which negatively affects both traditional and modeled coagulation properties (9,30).

CONCLUSION

Our study identified that milk components and clotting ability features were sullied by SCM. The high prevalence of SCM in cows and multi-drug resistance of the incriminated Staphylococci highlight regular monitoring of the disease at farm level. Every farm must have determined critical points of fresh milk production chain in their conditions. By continuous control of these critical points the possible hazards can be prevented, so the milk quality can be improved and maintained for the consumer confidence.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflict of interest

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