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ORIGINAL ARTICLE

Effect of the Season on the Microbiological quality of Raw cow's milk on the farm in Western Algeria

Elhachemi Sassi[1*], Sahnoune Attou[1], Abdelkader Homrani[1], Said Nemiche[1].
1- Laboratory of Sciences and Techniques of Animal Production, Abdelhamid Ibn Badis University, Mostaganem, Algeria.
***Corresponding Author**: hsassitaa_27@yahoo.fr

ABSTRACT
The microbiologic evaluation of raw milk in the west of Algeria during the four seasons (June 2015 to may 2016) was investigated in this paper. The pathogenic germs in milk were estimated and were compared according to Algerian norms; the lactic acid bacteria were counted on various culture medium: M17, MRS, MSE, PCAL, and a survey was conducted in parallel with the breeders to assess the quality of production practices. The study showed a significant difference in the presence of certain bacteria in the year. The total mesophilic flora, faecal coliforms and faecal streptococci were higher during the spring (3.8-10^5 cfu/ml, 4.1 10^3 cfu / ml, 3 10^2 cfu / ml), while the Staphylococcus aureus was almost stable even the higher contamination rate during the summer 40.74% for the infected samples. However, the presence rate of the clostridia was acceptable throughout the year and was found to be 66.66% in the autumn. The identification of the isolates lactic showed that the samples were dominated by streptococcus (37%) in summer sample, by enterococci (24%) in autumn, by lactococci (35%) in winter and by lactococci (30%) in spring. The physiological and biochemical analysis showed a diversity of dominant species between seasons; summer streptococcus thermophilus 33.7% (29 isolates), autumn enterococcus faecalis and leuconostoc lactis 14.2% (15 isolates) for both species, winter lactococcus lactis subsp lactis 16.2% (18 isolates) and spring lactococcus lactis subsp cremoris 11.4% (22 isolates). Moreover, the produced milk in the autumn and spring seasons were richest in lactic flora in relation to the conditions of production. In addition, the survey showed that the least hygienic practices were practiced during the winter and spring seasons for all farms.
Key words: raw milk, microbiology quality, lactic acid bacteria, seasons, west of Algeria.

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INTRODUCTION

Milk is a staple food for many mammals because it is a rich substrate of carbohydrates, lipids, vitamins and minerals; it is also used as a raw material in many products such as cheese. The hygienic conditions at the farm level and all along the production circuit until the arrival of the milk at the dairy has as many sources of contamination to control in order to preserve the hygienic quality of milk [1].

Algeria is the first consumer of milk in the Maghreb with 03 billion liters in the year [2], whose 02 billion are locally produced [3]. Algeria is the second importer of milk powder [4], with an average consumption of 100 liters/ inhabitant/ year. Milk sector was developed in Algeria with 08% [5], but this growth in the quantity produced was not accompanied with a satisfactory quality of milk due to unfavorable climatic conditions, the inadequate feeding, the lack of suitable installations and ignorance, the upkeep of unhealthy animals, which are obstacles reflecting on the quantity and quality of produced milk. However, some agents responsible for zoonoses can be transmitted to the human [6], and some cheese markers are suffering from this quality of milk which harms the healthiness cheese.

In Algeria, few works were reported on milk quality [7, 8] especially in the west of Algeria and at farm level.

In order to monitor food safety, it is imperative that the microbiological quality of milk be determined. Therefore, the objective of this work is to evaluate the bacteriological quality of raw milk in farms during

the four seasons on the one hand, and the other hand to study the diversity of these milks in flora of technological interest, flora to be used in many dairy and other fabrications, as well as to detect the upstream failures at the level of the farm, which can compromise the quality of the raw milk and constitute critical points to control.

MATERIAL AND METHODS

Presentation of study areas

The west of Algeria contains several areas of dairy vocation, the most important are; the city of Sidi Bel Abbes, the city of Relizane and finally the city of Mascara.

The province of Sidi Bel Abbès, with a strong agricultural vocation, covers a total area of 9150 km^2 in the north-western part of the country. It is located in the semi-arid continental bioclimatic stage was very hot and very rough winters; Spring and autumn are short. A dairy cattle farming is an important part of the agricultural economy of the province; its production makes of it, a dairy basin and class among the first dairy provinces. The total numbers of dairy cows are 21400, with a total number of collectors of 87 and 953 breeders. The total production of cow milk is estimated at 29 million liters. [9, 10].

The province of Relizane in the north-west of the country covers a total area of 4851.21$km^{2.}$ The climate of the province is continental cold rainy. A cattle breeding occupies an important place in the agricultural economy of the province. The total number of cows dairy and 22710 for a total number of collectors of 65 collectors and 605 breeders. The total production of raw milk is estimated at 70 million liters [9, 11].

The province of Mascara, finally, covers an area of 5135 km^2 in the north-western part of the country. Rainfall is on average 450 mm / year. The total number of cattle is 30 700 including 20 670 dairy cows and a total number of collectors of 78 collectors, as well as 812 breeders. The total production of raw milk is estimated at 68 million liters of milk. [9, 12]

Sampling procedure:

This study was carried out during June 2015 to May 2016, in the western region of Algeria. We chose 03 cities in this region; Mascara city, Sidi bel Abbes city and Relizane city. In this sectional study, sampling was done by selecting the farms with random, 03 farms per city were chosen. Raw milk was collected one time per month for each farm (total samples 108 = 1*3*3*4*3)

The samples were taken from a mixture milk (evening milking with that of the morning) in sterile vials, these samples were preserved at 6°C in an electric cooler [13] till analysis moment carried out after 4 hours.

Investigation:

At each visit, a survey was conducted by means of a survey on livestock management, the environment and habitat, the layout of premises, the distribution of food, and the conduct of milking. The aim was to identify the quality of existing breeding practices in the stables during the different seasons.

Microbiological analyzes

Search and enumeration of germs of contamination:

The bacteriological analyses were carried out according to Algerian official standards [14].

The intend examined germs were total mesophilic aerobic flora, faecal coliforms, faecal streptococci, *Straphylococcus aureus* and sulphite-reducing clostridiums.

Evaluation of aerobie mesophile flora was carried out on agar PCA (Institute Pasteur), incubation at 30°C for 48-72 hours [15] the colonies are enumerated and the result is expressed in colony-forming unit per ml of milk [16] .

faecal coliforms were detected on neutral red whey agar plate and violet crystal (VRBL) (Pasteur Institute Algeria), the incubation was done at 44 ° C for 24 hours, the red colonies with a diameter of 0.5 mm are counted [17].

Fecal streptococci were counted on Rothe medium (Institute Pasteur Algeria) as a presumptive culture and according to the most probable number method for incubation for 48 hours at 37°C. The turbid tubes will be seeded on BEA agar plates (esculin azide bile) as a confirmatory test for incubation at 37°C for 24 and 48 hours. All small translucent colonies surrounded by a black halo are considered faecal streptococci [18] .

Staphylococcus aureus was counted on Baird Parker agar (Institute Pasteur Algeria) supplemented with egg yolk and potassium tellurite, incubation at 37°C for 48 hours [18]. Gram, catalase and coagulase staining tests have been performed as confirmation tests [19]. *Staphylococcus aureus* occurs as black colonies with a clear halo and an opaque white border 0.5 to 2 mm in shiny aspect [20].

for sulphite-reducing clostridiums, the tubes containing the dilutions are subjected to heating at 80°C for 10 minutes to destroy the vegetative forms [21] and an immediate cooling to activate the clostridial spores. From these dilutions 5 ml are removed aseptically in a sterile tube supplemented with 7 ml of

liver agar (VF) (Institute Pasteur Algeria) previously added with an ampoule of iron alum and a sulphite sodium ampoule. After incubation at 37 ° C for 24 to 48 hours, the large and black colonies producing sulphides from the sulphites that precipitated with the iron ions are considered clostridia [22].
The interpretation of the results was made on the basis of the interministerial decree N ° 35 1998, the microbiological requirements for raw milk are; FTAM 105 cfu / ml, faecal coliforms 103ufc / ml, faecal staphylococci absence / 0.1 ml, CSF 50 cfu / ml, absence for *Staphylococcus aureus*.

Research and identification of lactic flora:

Obtaining isolates:

The elective and selective isolation of lactic acid bacteria culture on several media was performed according to the method described by [23]. In this method 1 ml of each dilution is inoculated in the solid media to obtain well-separation colonies.
Colonies were counted for each dilution to determine the number of cfu / ml. The short-term conservation of pure isolates is carried out on a MRS solid medium slant for lactic acid bacteria. After growth at the optimum temperature, the cultures are maintained at +4°C and re-plated every 4 weeks. The long conservation of the purified isolates is conducted in a specific medium containing 70% of skim milk enriched with 0.05% yeast extract and 0.05% glucose and 30% glycerol. Isolates were maintained at -80 ° C [24, 25, 26].

Choice of isolates:

From 1594 purified and examined isolates, 1269 isolates Gram positive, catalase negative and non- spore forming were retained. These latter were identified at the genus level. Only 444 isolates were identified at the species level by phenotypic methods.

Identification of isolates:

The identification of the isolates at the genus level was performed in two steps, the first being to test the isolates by Gram stain, catalase production and spore formation [27]. The second step was based on the morphological analysis (macroscopic and microscopic aspect) and type of fermentation. The microscopic observation in the fresh state made it possible to assess the morphology of the bacteria, their association and their mobility.
The identification of isolates is based on classical microbiology techniques based on the search for morphological, physiological and biochemical characters. These techniques have been described by [25, 28].
The identification of isolates at the species level has gone through a series of physiological and biochemical tests;
- Catalytic activity degrades hydrogen peroxyde into oxygen and water. It is demonstrated by emulsifying one or two colonies of the isolates in a fresh solution of hydrogen peroxide. Abundant gas evolution as foam reflects the decomposition of hydrogen peroxide by the action of the tested enzyme [29, 13, 26].
- Growth at different temperatures in MRS and M17 broth : 5, 10, 15, 37, 40 and 45 ° C, for 24 hours to 48 hours [25, 26, 30].
- Resistance test at 63.5 ° C for 30 min, then incubation at 30 ° C for 48h to 72h [25].
- Growth under hostile conditions with 2, 4 and 6.5% NaCl was observed in MRS and M17 broth at 30 ° C for 48h.
- The growth test in different pH environments is performed in MRS and M17 liquid media at pH 4.5, 5, 6, 6.5 and 9 the development is characterized by a disorder of the medium at the bottom end of the tube [25, 30].
- The production of gas is tested on MRS medium in tubes containing Dhuram bells to demonstrate the production of gas. After incubation at 37 ° C for 24h to 48h, the presence or absence of the gas in the bell indicates the fermentation type. The homo-fermentative strains will produce 90% lactic acid and only 10% CO_2, whereas the hetero-fermentative stains will produce lactic acid and CO_2 in equal proportions [23].
- Growth of bacteria on Sherman milk [24].
- Test of citrate; the medium does not contain citrate as the carbon source, only the bacteria with citrate permease are able to grow on this medium. The middle of the slant is seeded in a longitudinal groove by means of a handle containing a colony and the slant is incubated at 30°C for 5 days. A positive citrate result is manifested by alkalization of the medium (colour change of medium indicator to blue). A negative citrate result is manifested by the absence of bacterial growth (green colour, unchanged medium) [31, 32].
- Hydrolysis of arginine is tested on M16BPC medium after incubation at 37 ° C for 24h. a positive culture is identified by a change to yellow due to glucose metabolism. Degradation of arginine and the release of ammonia prevent the change to yellow [33, 26].

-Hydrolysis of esculin is tested on esculin-agar MR medium after incubation at 37 ° C. for 48 hours.
- Production of dextran is tested on GPY agar where glucose was replaced by sucrose and incubation for 5 days.
-Production of acetone is detected on medium Clark and Lubs. After incubation at 37°C for 24h, 1 ml of culture is deposited together with 0.5 ml of reagent a-naphthol in 6% of absolute alcohol (VP1) and 0.5 ml of a solution (NaOH 16% in distilled water (VP2)) in a haemolysis tube carry out the voges-proskaeur reaction. The tube is thoroughly stirred and kept in touch with the free area for 10 min at room temperature. The production of acetoin results in a pink ring on the surface of the yellow medium [31, 32, 34, 26].
- Mannitol mobility test; the bacterial stains were growed in mannitol mobility medium after incubation at 37°C for24h. Fermentation of mannitol resulted in a change of the culture medium to yellow [13].
- The fermentation of sugars is tested on specific media supplemented with a pH indicator (0.5% solution of chlorophenol red). All strains were also tested for fermentation of glucose, lactose, sucrose, maltose, L-arabinose, D-xylose, galactose, D-fructose, sorbitol, melibiose, D-raffinose, et mannose [35, 36, 32].

Statistical analysis:

The number of bacteria was expressed in colony forming units (cfu) per ml, the mean and standard deviation were calculated for each type of flora and each sample.
Data were analyzed by the STATISTICA version 2007 software. Descriptive statistics were established to report the variability of the different parameters involved in the evaluation of the milk hygienic quality. Then bacterial counts were transformed into logarithmic decimals and values equal to zero were converted to log 0.1. log transformed counts of microbiological indicators data were analyzed using factorial analysis of variance and Student test for comparison of differences between means of microorganism's number with respect to different sources and general linear model.

RESULTS

Hygienic and microbiological characteristics of raw milk:

During the four seasons the number of mesophilic aerobic germs was higher than the norm ($p < 0.01$). The lowest seasonal average was observed during the summer 1.6 10^5cfu/ml. this average has passed to 2 10^5cfu/ml, 3.4 10^5cfu/ml and 3.8 10^5cfu/ml during autumn winter and spring respectively. The comparison between the seasons for this germ showed a highly significant difference ($p < 0.01$). 74% of the samples were contaminated with this germ during the summer against 88%, 100%, 100% for autumn, winter and spring. The rate of contamination by this germ was significantly higher than the standard between the cities (tables 01, 02, 03, 04). The higher average of 2.5 10^5cfu/ml was observed for the city of Relizane and the lower average at the city of Sidi Bel Abbess 0.87 10^5 cfu/ml.
The number of fecal coliforms was significantly higher than the standard in raw milk samples reseived in winter and spring with a seasonal average of 4 10^3cfu/ml and 4.8 10^3cfu/ml. the levels of these germs in summer and autumn were 2.6 10^3 cfu/ml and 3 10^3cfu/ml respectively (table 05).all raw milk samples analyzed during the seasons were contaminated with these germs except those of summer where the rate contamination were 88%. The comparison between the cities did not show a significant difference during seasons. The highest average between cities was observed in raw milk of Relizane city during spring 5.2 10^3cfu/ml (table 04). The lowest average 1.2 10^3 was observed in Sidi Bel Abbess City during summer (table 01).
The number of fecal streptococci detected in raw milk samples analyzed in spring was very higher ($p < 0.01$) in comparison with those of other seasons 3 10^2cfu/ml. the comparison between the cities for this germ did not show a significant difference during the four seasons. The highest average was observed during spring in Mascara City 3.4 10^2cfu/ml (table 04) and the lowest average was observed during autumn in raw milk of Sdi Bel Abbess City 0.5 10^2cfu/ml (table 02).
The seasonal averages for *Staphylococcus aureus* were higher during the four seasons, but the comparison of the results did not show any significant difference (table 05). The highest contamination rate was observed in the summer samples 40%, 22% in autumn, 29% in winter and 37% in spring. The comparison of results between Cities did not show any significant difference. The lowest number was 0cfu/ml during autumn in raw milk of Mascara City and the highest number was 0.44 10^2cfu/ml during summer in raw milk of Relizane City (table 01).
The high concentartion of Clostridium sulfito-reducing was obtained during autumn 1.7 10^1cfu/ml higher ($p < 0.05$). The lowest number 0.2 10^1cfu/ml was obtained during spring (table 05) but these rates have not exceeded the norm. The comparison between Cities did not show any significant difference. The highest average 2 101cfu/ml was observed in Mascara City during autumn (table 02) and the lowest 0 cfu/ml in raw milk of Sidi Bel Abbess City during winter (table 03).

Lactic acid bacteria Results:

Lactic acid bacteria were counted in all milk samples analyzed. Isolates that do not exhibit the phenotypic characteristics of lactic acid bacteria (Gram positive, catalase negative and non spore-forming) were rejected. From 1594 isolates, 1269 were selected and are unequally distributed throughout the year; summer (247 isolates), autumn (299), winter (315), spring (408), from these results we note that the highest number of lactic isolates is obtained during spring coinciding with the period of strong lactation. The number in ufc / ml varies seasonally (Table 06) from 2.5 10^5 ufc / ml to 25.8 10^5 ufc / ml. We note that the highest values are recorded during the spring while the lowest average is obtained during the summer.

Microscopic observation of the isolates revealed that the form of the bacteria ranges from hulls, diplococci, chain hulls, tetrad hulls, oval hulls and coccobacilli to filamentous bacilli.

The remaining 1269 isolates were identified in genus level and only 444 isloates were identified in species by traditional phenotypic methods.

The 1269 isolates are divided into six groups; (1) round or lenticular white colonies, diplococci and in chain cells, thermophilics and homofermentative (presumptive streptococcus 174 isolates); (2) round or lenticular white colonies, cells, diplococci and in chains, mesophilic and homofermentative (presumptive lactococci 259 isolates); (3) round or lenticular white and brown colonies, long, coiled or filamentous bacilli or in small chains, small chain bacilli, homofermentative or heterofermentative (presumptive lactobacilli 126 isolates); (4) transparent colonies, cocci and oval chains [37], mesophilic, heterofermentative, argenine negative and growth at 6..5% Nacl (presumptive leuconostoc 264 isolates); (5) smooth rounded gray or whitish colonies, cocci in tetrads and homofermentative (presumptive pediococcus 176 isolates); (6) round or lenticular white colonies on M17 medium [38], diplococci and in chain cells [39], developing at 6.5% Nacl, pH 9.6 and a heat resistance at 63.5 ° C / 30 min (presumptive enterococci 270 isolates).

From 444 isolates identified, 53 isolates resemble *streptococcus thermophilus* by resistance at 63.5 ° C, growth at pH 6.5, not produce acetone, and not hydrolyze esculin. However, 08 isolates were identified as S*treptococcus bovis* by non-resistance at 63.5 ° C.

Ninety one isolates were identified as leuconostoc; 46 isolates belong to *L. lactis*, 37 isolates belong to *L. masentéroides* subsp *cremoris*, and 08 isolates to *L. mesenteroides* subsp *dextranicum* by their positive development at 37 ° C and their production of dextrane.

Enterococci; 93 isolates divided in 03 species; E*nterococcus durans* 24 isolates, which are mannitol negative and citrate negative, *Enterococcus faecalis* 56 isolates that were citrate positive, and 13 *Enterococcus faecium* that were citrate negative.

Ninety one isolates were identified as lactococci and were divided into 04 species; 44 isolates reunite with *lactococcus lactis* subsp *lactis*, which were citrate negative and acetoine negative, 29 isolates of *lactococcus lactis* subsp *cremoris*, which were acetoine positive, 15 isolates of *lactococcus plantarum* that were citrate negative and acetoine positive, and 03 isolates of *lactococcus rafinolactici* which were citrate negative, acetoine negative, heterofermentative and resisted to 63.5°C / 30 min.

The number of pediococci isolates was 63 divided in 04 species; 19 *Pediococcus acidilactici* which developed at pH 4.5, grow at 37°C and at 45°C and do not ferment maltose, 18 *pediococcus parvelus* developed at 37°C and not at 45°C and ferment maltose, .4 P*ediococcus pentasaceus* which were citrate positive, acetoine negative, resist at 63.5°C / 30 min, developed at 45°C and used maltose, 22 *Pediococcus damnosus* that do not develop at 45 ° C.

Forty five isolates were identified at Lactobacilli genus and were divided in 07 species; 14 isolates of lactobacillus acidophilus developed at pH 4.5, fermented lactose and sucrose, 04 *lactobacillus brevis* which developed at 10°C but not at 45°C, fermented lactose, sucrose, glucose and mannose, 10 *lactobacillus helviticus* , developed at 45°C, at pH 4.5 and fermented mannose, sucrose and lactose, *lactobacillus pentaseus* developed at 15°C and not at 45°C, fermented most of the sugars tested. 05 *lactobacillus plantarum* developed at 15°C and not at 45°C, fermented sugars and do not produce gas, 02 *lactobacillus casei* subsp *casei*, do not develop at 45°C, no gas production, 04 *lactobacillus para casei* subsp *para casei*, developed at 15°C and not at 45°C, no gas production and fermented mannose, lactose and sucrose.

DISCUSSION

The global microbiological characteristics of raw milk samples were unsafe to drink and were of poor quality. It indicated that the hygienic quality of raw milk differs from season to season and city to city.

Total aerobic mesophilic flora is a good indicator of contamination and provides information on the hygienic quality of raw milk [40]. The contamination can have several origins such as the skin of the animals, the hands of the caterer, the utensils of milking [41].

The high contamination was recorded during the spring season 3.8 10^5 ufc/ml and for the city of Relizane 5.1 10^5 ufc /. It indicated insufficient hygiene during milking and storage in farms. The low contamination was recorded during the summer season 1.6 10^5 ufc / ml and for the city of sidi belabbess 0.8 10^5 ufc/ml; for fear of the breeders towards the quality of the milk during this season. These results were lower than those of [20] 8.3 10^5 ufc/ml, a study conducted in winter, and than those reported by [42]10^7 ufc/ml in summer and lower throughout seasons to those of [2] 7.2 10^5 cfu / ml. And superior than those reported by [43], study conducted in winter in Morocco, 2.7 10^5 cfu / ml.

The quality of the milk we worked on is poor throughout the year, especially in the winter and spring. According to [44] they showed a lack of respect for good production practices and storage of milk. Therefore, mixing of fresh milk with that of the day before lead to high bacterial growth [45].

Table 01: Contamination rate during the summer season

Cities / Settings	Sidi bel abbes	Mascara	Relizane	Norms	Season effect
Aerobies Mesophiles Germs (CFU /ml)	$0.87\ 10^5 \pm 0.9\ 10^5$	$1.6\ 10^5 \pm 0.9\ 10^{5fg}$	$2.5\ 10^5 \pm 0.4\ 10^{5de}$	10^5	*
fecal Coliforms (CFU /ml)	$1.2\ 10^3 \pm 0.6\ 10^{3b}$	$1.9\ 10^3 \pm 0.8\ 10^{3b}$	$4.8\ 10^3 \pm 3.6\ 10^{3b}$	10^3	ns
Fecal Streptocoques (CFU /ml)	$0.8\ 10^2 \pm 0.9\ 10^{2c}$	$1.2\ 10^2 \pm 0.510^{2c}$	$0.8\ 10^2 \pm 0.6\ 10^{2c}$	Abs/0.1 ml	ns
Staphylococcus aureus (CFU /ml)	$0.28\ 10^2 \pm 0.44\ 10^2$	$0.16\ 10^2 \pm 0.26\ 10^2$	$0.44\ 10^2 \pm 0.46\ 10^2$	Abs	ns
Clostridium sulfito-reducer (CFU /ml)	$0.3\ 10^1 \pm 0.7\ 10^{1b}$	$0.6\ 10^1 \pm 1\ 10^{1b}$	$0.7\ 10^1 \pm 1\ 10^{1b}$	50	ns

Each group is represented by a number of repetitions n = 27; the results are expressed in mean values followed by the corresponding standard deviations; **: highly significant effect (p <0.01) of the factor studied *: Significant effect (p <0.05) of the factor studied; NS: non-significant effect (p> 0.05) of the factor studied; A, b, c: statistical comparison between the averages two at two by the test of Newman

Table 02: Contamination rate during the autumn season

	Autumn				
Cities / Settings	Sidi bel abbes	Mascara	Relizane	Norms	Season effect
Aerobies Mesophiles Germs (CFU /ml)	$1.2\ 10^5 \pm 3.9\ 10^{5g}$	$2.4\ 10^5 \pm 4\ 10^{5de}$	$2.5\ 10^5 \pm 3.3\ 10^{5d}$	10^5	*
fecal Coliforms (CFU /ml)	$2\ 10^3 \pm 1.1\ 10^{3b}$	$2.7\ 10^3 \pm 1.2\ 10^{3b}$	$4.3\ 10^3 \pm 1\ 10^{3b}$	10^3	ns
Fecal Streptocoques (CFU /ml)	$0.5\ 10^2 \pm 0.5\ 10^{2c}$	$1.4\ 10^2 \pm 0.5\ 10^{2c}$	$1\ 10^2 \pm 0.6\ 10^{2c}$	Abs/0.1 ml	ns
Staphylococcus aureus (CFU /ml)	$0.38\ 10^2 \pm 0.69\ 10^2$	0	$0.38\ 10^2 \pm 0.69\ 10^2$	Abs	ns
Clostridium sulfito-reducer (CFU /ml)	$1.5\ 10^1 \pm 1.5\ 10^{1a}$	$2\ 10^1 \pm 1.5\ 10^{1a}$	$1.6\ 10^1 \pm 1.6\ 10^{1a}$	50	ns

Each group is represented by a number of repetitions n = 27; the results are expressed in mean values followed by the corresponding standard deviations; **: highly significant effect (p <0.01) of the factor studied; *: Significant effect (p <0.05) of the factor studied NS: non-significant effect (p> 0.05) of the factor studied; A, b, c: statistical comparison between the averages two at two by the test of Newman

Table 03: Contamination rate during the winter season

	Winter				
Cities / Settings	Sidi bel abbes	Mascara	Relizane	Norms	Season effect
Aerobies Mesophiles Germs (CFU /ml)	$1.7\ 10^5 \pm 5.9\ 10^{5ef}$	$3.7\ 10^5 \pm 7\ 10^{5c}$	$4.7\ 10^5 \pm 6.1\ 10^{5ab}$	10^5	*
fecal Coliforms (CFU /ml)	$3.5\ 10^3 \pm 0.5\ 10^{3a}$	$3.5\ 10^3 \pm 0.7\ 10^{3a}$	$5\ 10^3 \pm 0.7\ 10^{3a}$	10^3	ns
Fecal Streptocoques (CFU /ml)	$1.7\ 10^2 \pm 0.9\ 10^{2b}$	$2.5\ 10^2 \pm 1\ 10^{2b}$	$2.1\ 10^2 \pm 0.3\ 10^{2b}$	Abs/0.1 ml	ns
Staphylococcus aureus (CFU /ml)	$0.14\ 10^2 \pm 0.33\ 10^2$	$0.33\ 10^2 \pm 0.5\ 10^2$	$0.27\ 10^2 \pm 0.44\ 10^2$	Abs	ns
Clostridium sulfito-reducer (CFU /ml)	0	$0.7\ 10^1 \pm 1\ 10^{1b}$	$1.7\ 10^1 \pm 0.8\ 10^{1b}$	50	ns

Each group is represented by a number of repetitions n = 27; the results are expressed in mean values followed by the corresponding standard deviations; **: highly significant effect (p <0.01) of the factor studied *: Significant effect

(p <0.05) of the factor studied NS: non-significant effect (p> 0.05) of the factor studied A, b, c: statistical comparison between the averages two at two by the test of Newman

Table 04: Contamination rate during the spring season

Spring					
Cities / Settings	Sidi bel abbes	Mascara	Relizane	Norms	Season effect
Aerobies Mesophiles Germs (CFU /ml)	$2.0\ 10^5 \pm 7.6\ 10^{5de}$	$4.3\ 10^5 \pm 6.1\ 10^{5bc}$	$5.1\ 10^5 \pm 3.2\ 10^{5a}$	10^5	*
fecal Coliforms (CFU /ml)	$3.4\ 10^3 \pm 0.8\ 10^{3a}$	$3.6\ 10^3 \pm 0.3\ 10^{3a}$	$5.2\ 10^3 \pm 0.6\ 10^{3a}$	10^3	ns
Fecal Streptocoques (CFU /ml)	$2.7\ 10^2 \pm 0.5\ 10^{2a}$	$3.4\ 10^2 \pm 0.6\ 10^{2a}$	$2.8\ 10^2 \pm 0.3\ 10^{2a}$	Abs/0.1 ml	ns
Staphylococcus aureus (CFU /ml)	$0.27\ 10^2 \pm 0.44\ 10^2$	$0.13\ 10^2 \pm 0.2\ 10^2$	$0.26\ 10^2 \pm 0.35\ 10^2$	Abs	ns
Clostridium sulfito-reducer (CFU /ml)	$0.3\ 10^1 \pm 0.7\ 10^{1b}$	$0.1\ 10^1 \pm 0.3\ 10^{1b}$	$0.4\ 10^1 \pm 0.8\ 10^{1b}$	50	ns

Each group is represented by a number of repetitions n = 27; the results are expressed in mean values followed by the corresponding standard deviations; **: highly significant effect (p <0.01) of the factor studied; *: Significant effect (p <0.05) of the factor studied; NS: non-significant effect (p> 0.05) of the factor studied; A, b, c: statistical comparison between the averages two at two by the test of Newman

Table 05: Seasonal variations of the microbiological quality of raw cow's milk collected in western Algeria

Seasons / Settings	Summer	Autumn	Winter	Spring	Norms	Season effect
Aerobies Mesophiles Germs (CFU /ml)	$1.6\ 10^5 \pm 0.7\ 10^{5d}$	$2.0\ 10^5 \pm 3.6\ 10^{5c}$	$3.4\ 10^5 \pm 6.1\ 10^{5b}$	$3.8\ 10^5 \pm 5.7\ 10^{5a}$	10^5	**
fecal Coliforms (CFU /ml)	$2.6\ 10^3 \pm 2\ 10^{3b}$	$3\ 10^3 \pm 1.1\ 10^{3b}$	$4\ 10^3 \pm 0.6\ 10^{3a}$	$4.1\ 10^3 \pm 0.6\ 10^{3a}$	10^3	**
Fecal Streptocoques (CFU /ml)	$0.9\ 10^2 \pm 0.7\ 10^{2c}$	$1\ 10^2 \pm 0.5\ 10^{2c}$	$2.1\ 10^2 \pm 0.7\ 10^{2b}$	$3\ 10^2 \pm 0.5\ 10^{2a}$	Abs/0.1 ml	**
Staphylococcus aureus (CFU /ml)	$0.3\ 10^2 \pm 0.38\ 10^2$	$0.25\ 10^2 \pm 0.54\ 10^2$	$0.25\ 10^2 \pm 0.41\ 10^2$	$0.22\ 10^2 \pm 0.33\ 10^2$	Abs	ns
Clostridium sulfito-reducer (CFU /ml)	$0.5\ 10^1 \pm 0.9\ 10^{1b}$	$1.7\ 10^1 \pm 1.5\ 10^{1a}$	$0.8\ 10^1 \pm 0.7\ 10^{1b}$	$0.2\ 10^1 \pm 0.6\ 10^{1b}$	50	*

Each group is represented by a number of repetitions n = 27; the results are expressed in mean values followed by the corresponding standard deviations; **: highly significant effect (p <0.01) of the factor studied; *: Significant effect (p <0.05) of the factor studied; NS: non-significant effect (p> 0.05) of the factor studied

A, b, c: statistical comparison between the averages two at two by the test of Newman

Table 06: Incidence of lactic acid bacteria during the seasons

Seasons	Summer	Autumn	Winter	Spring
Ufc/ml	$2.5\ 10^5$	$4\ 10^5$	$9\ 10^5$	$25.8\ 10^5$
Number of isolates	247	299	315	408
Cells form	Bacilli 11 % Hull 89 %	Bacilli 17 % Hull 83 %	Bacilli 12 % Hull 88 %	Bacilli 02 % Hull 98 %
Genus related	*Streptococcus* 37 % *Enterococcus* 19% *Leuconostoc* 23 % *Lactobacillus* 11 % *Pediococcus* 10%	*Enterococcus* 24 % *Streptococcus* 13 % *Lactobacillus* 18 % *Leuconostoc* 20 % *Pediococcus* 16 % *Lactococcus* 09 %	*Enterococcus* 17 % *Leuconostoc* 20 % *Lactobacillus* 12 % *Lactococcus* 35 % *Pediococcus* 11% *Streptococcus* 05%	*Enterococcus* 24 % *Leuconostoc* 20 % *Streptococcus* 07 % *Pediococcus* 17 % *Lactococcus* 30 % *Lactobacillus* 02 %

Table 07: Environments used and isolation conditions of lactic acid bacteria strains

Micro-organisms	Community isolation	Temp. C°	Duration	Incubation	Macro-morphology	Micro-morphology
Enterococci	M17 [79]	45	72	Aérobisis	White colonies round or lenticular	Coccis diplocoques and chains
Lactic Streptococci	M17 [38]	45	24-72	Aérobisis	White colonies round or lenticular	Coccis diplocoques and chains
Pédiococci	M17 [79]	30	48-72	Aérobisis	Smooth colonies rounded grayish or whitish	Long and short chain tetrad
Lactococci	PCAL [73]	30	48-72	Aérobisis	White colonies round or lenticular	Coccis diplocoques and chains
Leuconostoc	MSE [65]	25	48-72	Aérobisis	Transparent colonies very small and round	Oval coccis in chains
Lactobacilli	MRS [80]	37	24-72	Anaérobisis	Small white colonies round or lenticular	Small sticks and in chains

Table 08: physiological and biochemical characteristics of isolates stains

Characteristics		Stains																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Growth at temperature (C°)																								
	10	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
	15	-	+	+	-		-	+	+	-	-	+	+	+	+	+	-	+	-	-	+	+	+	+
	37	+	+	-		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	-		+
	45	+	+	-	+	+	-	-	+	+	+	-	-	-	v	-	-	+	-	-	-	-	-	-
Growth in medium with NaCl %	2	v	+	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	-
	4	-	+		-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	-	-
	6.5	-	+	-	+	+	-	-	-	-	-	+	-	+	-	-	-	v	+	-	-	-	-	+
Growth at pH	4.5	-	-	-		-	-	-	-	+	+	-	+	+	+	-	-	+	+	-	-	+	-	-
	6	+	+	-		-				+	-	+	+	+		+	-	+	-	-	+			
	6.5	+	+	-		-	+	+	+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	+
	9	+	+	+	-	+	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+	
Production of CO_2		-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Heat resistance 63.5°C/ 30'		+	-	v	+	+	+	-	-	-	+	+	-	-	-	+	-	+	-	-	v	-	-	-
Esculin hydrolysis																								
Citrate hydrolysis		-	-	+	ND	ND	-	-	v	+	-	-	v	+	+	-	-	+	-	-	+	+	v	-
ADH production		-	+	-	+	-					-	-	-	-	-	-	-	-	-	+	-	-	-	-
Acetoin production		-	-				-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
Dextran production		-	+	-	+	+	+	+	+	-	-	v	-	+	-	+	v	+	-	-	-	+	+	+
Mannitol fermentation		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
		-	+	-	+	+					+	+	-	+	-	+	-	-	-	-	+	-	+	-

1. Streptococcus thermophilus 2.Streptococcus bovis 3. Enterococcus Durans 4.Enterococcus Faecalis 5.Enterococcus Faecium 6.Leuconostoc Lactis 7.Leuonostoc. Mesteroïdes subsp cremoris 8.Leuconostoc Mesteroïdes subsp dextarnicum 9.Lactobacillus Acidophilus 10.Lactobacillus Helviticus 11.Lactobacillus Pentaseus 12.Lactobacillus Brevis 13.Lactobacillus Plantarum 14.Lactobacillus Casei subsp casei 15.Lactobacillus Para casei subsp para casei 16.Pediococcus damnosus 17. Pediococcus acidilactici 18..Pediococcus parvulus 19.Pediococcus pentasaceus 20.Lactococcus Lactis subsp lactis 21.Lactococcus Plantarum 22.Lactococcus Lactis subsp cremoris 23.Lactococcus Rafinolactis.

(+) positive reaction, (-) negative reaction, (v) more than 10% and less than 90% of positive reaction, ND no determination, ADH argentine dihydrolase

The presence of fecal coliforms in milk indicate recent faecal contamination, because these bacteria cannot survive outside the intestine for a long time [8, 43], The presence of a high numbers of coliforms in milk provides a hygienic quality index used in the production of milk, contaminated udders and teats may contribute to the presence of coliforms from various sources such as manure, soil, food, personnel and even water [46], as they can lead to food poisoning [47]. In our study the low mean seasonal was 2.6 10^3 ufc / ml in summer and the highest was 4.1 10^3 ufc / ml in spring, the city of Relizane has the highest average 5.2 10^3 ufc/ml, and the lowest for the city of sidi bellabess 1.2 10^3 ufc /ml. these results were higher than those of [48] 1.7 10 ufc/ml and lower than those of [49] 2 10^6 ufc/ml and [50] 3.2 10^5 ufc /ml and significantly lower than those of [43] 4.2 10^7 ufc /ml in Morocco.

The average fecal streptococci burden was variable during the year, with the highest recorded during the spring 3 10^2 ufc / ml, followed by winter 2.1 10^2 ufc / ml and 3.4 10^2 ufc / ml for the city of Mascara, these

results are superior to those of [8] 0.51 10^2 ufc/ml and lower than [50] 4 10^4 ufc/ml. The lowest averages are 0.9 10^2 ufc / ml during the summer season and 0.5 10^2 ufc/ ml for the city of sidi belabess during the autumn season. They are indicators of faecal contamination and unhygienic handling [2], the main vectors are teats, skin and poorly cleaned milking equipment[51].

Table 08a: physiological and biochemical characteristics of isolates stains

Characteristics	Stains																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Acid production from																							
Xylose	-	-	+	-	-	-	-	+	+	-	+	+	+	+	-	-	+	-	+	+	-	-	+
Galactose	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+
Glucose	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	+	+	-	+	-
Mannose	+	-	-	-	-	+	-	+	-	-	-	+	+	+	-	-	-	+	-	-	+	-	+
Mannitol	-	-	-	+	+	-	-	+	-	-	-	+	-	+	+	-	-	-	+	-	+	-	-
Sorbitol	-	-	-	+	+	-	-	+	-	-	-	+	-	+	+	-	-	-	+	-	+	-	-
Cellobiose	-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-
Maltose	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-
Lactose	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-
Melibiose	-	+	ND	ND	ND	+	-	+	-	-	+	+	+	+	+	+	-	-	+	-	-	+	+
Sucrose	+	+	+	ND	+	+	-	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+
Raffinose	-	+	-	-	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	-	-	+	+

Table 09: Distribution of lactic acid bacteria genus types over the four seasons

	Summer		Autumn		Winter		Spring	
	N	%	N	%	N	%	N	%
Streptococci	91	37	38	13	16	05	29	07
Enterococci	47	19	72	24	53	17	98	24
Leuconostoc	57	23	61	20	63	20	83	20
Lactobacilli	27	11	53	17	38	12	08	02
Pediococci	25	10	48	16	35	11	68	17
Lactococci	00	00	27	09	110	35	122	30

N ; number of isolates, % ; percentage on all isolates.

Table 10: Distribution of lactic acid bacteria species over the four seasons

	N	%	Summer	Autumn	Winter	Spring
Streptococcus thermophilus	53	11.90	29	13	01	10
Streptococcus bovis	08	01.80	03	00	05	00
Ent. Durans	24	05.40	05	07	03	09
Ent. Faecalis	56	12.60	11	15	15	15
Ent. Faecium	13	02.90	00	03	00	10
Leuc. Lactis	46	10.36	14	15	02	15
Leuc. Mesteroïdes subsp cremoris	37	08.33	06	06	11	14
Leuc. Mesteroïdes subsp dextarnicum	08	01.80	00	00	08	00
Lb. Acidophilus	14	03.15	03	06	03	02
Lb. Helviticus	10	02.25	04	03	03	00
Lb. Pentaseus	06	01.35	02	00	04	00
Lb. Brevis	04	00. 67	00	03	00	00
Lb. Plantarum	05	01.12	00	05	00	00
Lb. Casei subsp casei	02	00.45	00	02	00	00
Lb. Para casei subsp para casei	04	00.90	00	00	04	00
Pediococcus damnosus	22	04.95	09	04	05	04
Pediococcus acidilactici	19	04.27	00	09	00	10
Pediococcus parvulus	18	04.05	00	04	04	10
Pediococcus pentasaceus	04	00.90	00	00	04	00
Lact. Lactis subsp lactis	44	09.90	00	06	18	20
Lact. Plantarum	15	03.37	00	04	11	00
Lact. Lactis subsp cremoris	29	06.50	00	00	07	22
Lact. Rafinolactis	03	00.67	00	00	03	03
Total	444	99.59	86	105	111	142

Ent ; enterococcus, leuc ; leuconostoc, Lb ; lactobacillus, Lact ; Lactococcus
N : number of species. % : percentage on all species

Staphylococcus aureus can produce enterotoxins responsible for food poisoning, they can gain raw milk either directly by excretion in infected neighborhoods in case of clinical or subclinical infection or indirectly by the environment during handling and processing operations of raw milk [52, 53, 50], they are excreted in milk with a wide fluctuation from 0 to 10^8 ufc / ml, *Staphylococcus aureus* is considered the third most important cause of disease in the world among the reported food borne illnesses [54].

The comparison of the results obtained during the 04 seasons and in the 03 cities did not show a significant difference ($p > 0.05$) for this germ, according to our results the rates of the infected samples during the summer season, the autumn, winter and spring are respectively 40.74%, 22.22%, 29.62%, 37.03%. The highest seasonal average is recorded during the summer season with a rate of 0.3 10^2 ufc / ml and the lowest is from 0.22 10^2 to 10^2 ufc / ml during the spring. The high load was at the city of Relizane l with a rate of 0.44 10^2ufc / ml during the summer and the lowest was 0 at the Mascara area during the fall. It has been shown that when the level of contamination exceeds 10^3 bacteria / ml, on average 25% of cows are infected, these results are lower than those of [43, 8] with an average load 6 10^2 ufc / ml, [2] 0.9 10^3 ufc / ml, [50] 8 10^4 ufc / ml and [55] 1.2 10^6 ufc / ml.

. The Algerian standard for Clostridium sulfito-reducer is 50 germs / ml, the presence of these anaerobes reflects contamination recent or old, fecal or soil-borne, clostridiums perfringens is sometimes suspected [22], the results of this study show that no sample has exceeded the standard nevertheless there are contamination rates that differ from one season to another and from one city to another, contamination rates during the summer season , autumn, winter and spring are respectively 33.33%, 66.66%, 44.44%, 18.51%, the highest seasonal average load is 1.7 10^1 ufc / ml for the fall season, this average is lower than that reported by [2] 2.7 10^1 ufc / ml and higher than that reported by [56] 0.4 10^1 ufc / ml. [20] showed that 29.4% of the samples are contaminated, [2] showed a rate of contamination 12.5%. The lowest seasonal average was 0.2 10^1 ufc / ml during the spring, the highest average load in this flora was 2 10^1 ufc / ml in the city of Mascara during the autumn, according to [57] butyric spores increase from barn entry and that butyric contamination is mainly due to the presence of soil in hay, and the use of grass silage in winter.

. We note from the results that there is a significant difference ($p > 0.05$) when comparing the results for all enumerated bacteria, except *Staphylococcus aureus*, during the 04 seasons and in the 03 cities, the same observation was observed for the farm-level survey, these results showed that there was a difference in livestock farming practices especially between seasons and a certain similarity between the cities during the investigation period. It appears that basic milking practices have been much more practiced during the summer season, namely the rapid cooling of milk after milking and the immediate cleaning of milking utensils, for fear of on the part of the breeders towards the quality of these milks, the thing completely absent during the cold period, which gives the possibility to the biofilms to developed on the surface of milking equipment. [58] showed that the milk is enriches during the passage in the milking machine in flora alteration.

It is noted that from the mid-winter period when the animals calved and during the spring season, a period of high lactation, less attention was paid to the animals at that time by the breeders, the same finding has been observed by [57].

In addition, the quality of the litter used by the animals was of poor quality, for all farms, during the winter and spring seasons, whereas in summer 55% of farms do not use litter and 45% use In fresh litter, it was observed that the microbial load of a litter used by cows was higher than that of fresh litter. The coliforms, streptococci and staphylococcus population levels increased with variations ranging from 10 to 10^6 according to microorganisms and materials used as litter [59, 60].

It was noted that the number of lactic acid bacteria varied seasonally (Table 05) from 2.5 10^5 ufc/ml to 25.8 10^5 ufc/ml. this was related to the conditions of milk production at the farm level. These results are lower than those obtained by [32, 28]

The microscopic observation showed that the hull form was dominant throughout the year in summer 89%, autumn 83%, winter 88%, spring 98%, the same result is brought by [61] and [32] . The bacilli form was present with variable percentages 11%, 17%, 12%, 02% during the summer, autumn, winter and spring respectively, this was due to farming practices especially distributed feeding during the seasons. Our results are in agreement with those of [62].

Distribution of lactic acid bacteria:

The results of distribution of genera (table 09) between the 04 seasons showed a clear dominance of streptococcus during the summer (37%) followed by leuconostoc (23%) and then enterococci (19%), in fourth position lactobacillus (11%) and finally the pediococci (10%). During the autumn the distribution of lactobacillus and pediococci was seminal (18% and 16%), the streptococci and pediococci decreased to

13% and 20% respectively, the enterococci increased to 24% and lastly the lactococci 07% which was absent during the summer.

For the winter period the distribution was heterogeneous between the genera; lactococci was the dominant genus (35%), streptococci decreased with enterococci, 05%, 17% respectively, lactobacillus 12%, and pediococci 11%, while leuconostoc were stable during autumn, winter and spring (20%).

The spring season that coincides with the period of high lactation has demonstrated heterogeneity of distribution between different genera with a dominance of lactococcus (30%), the most remarkable is the passage of lactobacillus from 12% during the winter to 02% only during the spring.

Enterococci are used to improve the taste quality of cheddar cheese and other cheeses. The enterococci genus is present throughout the year with variable frequencies (19%, 24%, 17%, and 24%). These results are in agreement with those of [63] which showed a strong predominance of genus enterococci, while our results are superior to those reported by [32] where this genus was represented by only 02 strains. The presence of the enterococci genus in our study with variable frequencies is probably due to the hygienic conditions of milking and storage of milk on the farm which were variable during the four seasons.

Leuconostocs are used as flavor leavens in order to improve the structure of cheeses and to eliminate certain taste defects [64]. These microorganisms develop on MSE medium [65] and at a temperature between 18 ° C to 30 ° C, also have a tolerance to variations in concentrations, the incubation was done in aerobic [28]. The rate of leuconostocs was the most stable between seasons (20%), however we recorded a drop of 23% during the summer to 20% for the other seasons. This differentiation is to be explained, it may be due to the composition of the milk of each race exploit from one farm to another [66]. [25] found that the highest leuconostoc level was determined in the Arabia (goat) race. [32] found heterogeneity in the distribution of lactic acid bacteria, leuconstoc was the most common genus found on the 04 desired genera. The origins of leuconostoc, which may constitute sources of contamination by these microorganisms, are mainly silage used for feeding animals. [67] Isolated *leuconostoc mesenteroids* from corn silage and alfalfa samples taken from farms in the Emilia-Romagna region.

The streptococcus genus was present during the four seasons, the highest rate was recorded during the summer (37%) then this rate began to decrease 13% (fall) up to 05% (winter) and 07% (spring). Isolates belonging to this genus developed at pH 6.5 at 45 ° C, and resistance at 63 ° C (others not), did not develop at 4% and 6.5% NaCl (others Are growing). [32] revealed a presence of 14% of all the isolates studied, while [25] found 11% (46 isolates) from goat's milk.

The rate of lactobacillus is variable from one season to another. The highest rate is obtained during autumn (18%) followed by winter (12%) then summer (11%) and the lowest (02%) in spring. We recorded a complete absence of this type in most of the samples analyzed during spring. A similar result is obtained by [62, 68, 69] where lactobacilli were twice as high in winter than in summer. These authors explain these variations by the practices of breeding and in particular the use of hay, free or impassable stabling and hygiene. It appears that the use of hay and the presence of hay in the litter are associated with higher levels of lactobacilli [70, 62]. In our case and according to the survey result we noticed the use of hay in most of the farms studied during the autumn and winter, hence the high rate of lactobacilli and almost the absence of this practice during the spring (use of pasture) hence the lowering of lactobacilli.

The genus Pediococci was determined during the summer, autumn, winter and spring (10%, 16%, 13%, 17%) respectively. The highest rate was obtained during the spring (17%) and the lowest rate (10%) during the summer. This is in relation with the farming conditions at the farm level. The genus pediococci is in the form of gray-white or whitish rounded colonies on M17 medium. Microscopic observation revealed the presence of hulls in tetrads. Isolates belonging to this genus showed positive development at 37 ° C, at pH6, some strains developed at 45 ° C others not. The composition of lactic acid bacteria is related and depends mainly on the material on which the isolation has been carried out [71].

The lactococci genus was the dominant genus during winter (35%) and spring (30%), it was 09% during the autumn and totally absent during the summer. Lactococci can be isolated from plant products, which are probably their main reservoir [72]. The most appropriate explanation for their absence during the summer is perhaps the absence of vegetation during this season. This genus develops at low temperatures, at pH 6.5, not at 45 ° C and is homofermentative. The number of isolates during autumn, winter and spring are respectively 27, 110, 98 isolates, [25] found 90 isolates, [32] and [36] 52 isolates.

The distribution of species between the 04 seasons (Table 10) was heterogeneous; summer (86 isolates), autumn (105 isolates), winter (111), spring (142). The summer season was dominated by *Streptococcus thermophilus* (29 isolates) followed by *leuconostoc lactis* (14 isolates), *enterococcus faecalis (*11 isolates), *pediococcus damnosus* (09 isolates), *leuconostoc mesenteroids* subsp *cremoris* (06 isolates).), *enterococcus*

durans (05 isolates), *lactobacillus helviticus* (04 isolates), *lactobacillus acidophlus* and *streptococcus bovis* (03 isolates) for each species and last *lactobacillus pentaceus* (02 isolates).
The season of autumn was characterized by the presence of 11 differents species distributed in this way; *leuconostoc lactis* and *enterococcus faecalis* had the same number of isolates (15 isolates) followed by *streptococcus thermophilus* (16 isolates), *pediococcus acidilactici* (09 isolates), *enterococcus durans* (07 isolates). Lactobacillus was represented by 05 species; *lactobacillus acidophilus* (06 isolates), *Lb. Plantarum* (05 isolates), *Lb. Brevis* (03 isolates), *Lb. Helviticus* (03 isolates), Lb. *Casei subsp casei* (02isolates*). Leuconostoc mesenteroids* subsp *cremoris* (06 isolates), *lactococcus lactis* subsp *lactis* (06 isolates), *lactococcus plantarum*, *pediococcus damnosus*, *pediococcus parvelus* (04 isolates) for each species and *enterococcus faecium* (03 isolates).
The winter season was characterized by the presence of 18 different species distributed in this way; the predominance was for *lactococcus lactis* subsp *lactis* (18 isolates) followed by *enterococcus faecalis* (15 isolates), *lactococcus plantarum* and *leuconostoc mesenteroids* subsp *cremoris* (11 isolates) for each species, *leuconostoc dextranicum* (08 isolates) this species was present only during the winter, *lactococcus lactis* subsp *cremoris* (07 isolates), the other species were present at a lower number between 05 and 02 isolates. Lactobacilli was present in 04 species; *lactobacillus pentaseus* (04 isolates), *Lb. Paracasei* subsp *paracasei* (04 isolates), *Lb. Acidophilus* and *Lb. Helviticus* (03 isolates) for each species. Some authors have put forward the hypothesis that a planting of flora of technological interest (lactococci and leuconostoc) and acidifying flora can be made from litter type straw and teats to join the milk [73, 58, 74] .
Concerning the spring season, the predominance was in favor of *lactococcus lactis* subsp *cremoris* (22 isolates) and *lactococcus lactis* (20 isolates) followed by *leuconostoc lactis* (15 isolates), *enterococcus faecalis* (15 isolates), *leuconostoc mesnteroid* subsp *cremoris* (14 isolates), *streptococcus thermophilus* (10 isolates). We note that during this season the number of lactobacilli decreased to only 03 isolates; *Lb. Acidophilus* (02 isolates), *Lb. Brevis* (01 isolate). The spring season was characterized by high milk production, the use of abundant vegetation and almost the absence of the use of hay, this may be the cause of this notable fall in lactobacilli. *Lactococcus lactis* species were in 42 isolates and were the dominant during the spring, this is related to milking conditions, the environment of animals and conditions related to this season such as temperature, vegetation. *Lactococcus lactis* are frequently found on the surface of cow teats [75, 73, 70].
This diversity of genera and species from one season to another and from one farm to another is related to farm-level practices that were different from one season to another as shown by the investigation. This diversity is also observed for goat's milk. [76, 77, 78] have shown that species diversity is related to the raw milk composition of each goat breed and this is probably due to the environment of the animals.

CONCLUSION

The present study, which is part of an investigation that covers the whole chain of production of milking until the arrival at the dairy, has shown that the sanitary quality of milks was below the norm and varies at During the course of the year, these variations are related to production practices at the farm level, the lack of respect for these practices is at the basis of this variation.
The presence of pathogenic bacteria in milk during all seasons can be a serious problem for the health of the consumer. The study of the diversity of milks in lactic flora showed a significant diversity between the seasons due mainly to the conditions of production and the composition of these milks which varies from one season to another. The milk of the summer season is the least contaminated, at the farm level, and the least rich in lactic flora, during this season the most hygienist practices were practiced for fear of the breeders, and the richest in lactic flora is that of autumn and spring during which lactic acid bacteria are more frequented than other seasons. The hygienic and technological quality of raw milk for milking can be improved by the installation of technical support for farmers and the maintenance of good production practices throughout the year and the selection of certain practices which enriched milk by lactic flora. Thus, according to[74], cow milks rich in flora of technological interest and poor in coagulase-positive staphylococci are rather associated with hygiene practices considered "moderately" or even "unsafe". The season can influence the levels of the harmful or harmful bacteria through production practices, distributed feeding and vegetation. In perspective, 1- it would be interesting to confirm the identity of strains by molecular biology techniques. 2- to study the technological abilities of these strains for use in the manufacture of cheeses and fermented milks.

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