COMPARATIVE STUDY OF THE BACTERIAL FLORA OF MILK OBTAINED BY MANUAL AND MECHANICAL MILKING IN THE REGION OF OUM EL BOUAGHI

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Abstract
Bivalve shellfish is considered as delicious and healthy food items in several dietary regimes in the Niger Delta but the The objective of the present study is to compare the microbiological quality between the milks obtained by the two milking methods mechanical and manual. Twenty samples of raw milk were the subject of our microbiological investigations involving ten floras. Through this research, we assessed the degree of contamination of the raw material, cow's milk.Microbiological analyses of the samples of the milk studied show the complete absence of salmonella, Clostridium, yeasts and molds and fecal streptococci in milk obtainedbyboth mechanical and manual methods.However, the milk samples show an average contamination of the total aerobic mesophilic flora of $1.8\times10^{7}\pm 0.5\times10^{7}$CFU/ml for manual milking and $1.44\times10^{7}\pm 0.66\times10^{7}$CFU/ml for mechanical milking, total coliforms of $2.1\times10^{4}\pm 2.40\times10^{4}$CFU/ml for mechanical milking and absence for manual milking, fecal coliforms of $0.69\times10^{4}\pm 1.06\times10^{4}$CFU/ml for mechanical milking and absence for manual milking, Staphylococci of $0.93\times10^{3}\pm 0.77\times10^{3}$CFU/ml and the absence in milk obtained by mechanical milking and lactic bacteria of $5.12\times10^{4}\pm 4.07\times10^{4}$CFU/ml for mechanical milking and $0.7\times10^{4}\pm 1.33\times10^{4}$CFU/ml for manual milking.Raw cow's milk obtained by manual milking has a relatively good microbiological quality and is acceptable from a hygienic point of view. The absence of salmonella, streptococci and Clostridium indicates good cow health and good milking hygiene.

Key words: raw milk, microbiological quality, manual milking, mechanical milking, Oum El Bouaghi.

Received: 22.12.2019 Reviewed: 26.02.2020 Accepted: 02.03.2020

INTRODUCTION

Algeria is the largest consumer of milk in the Maghreb, with nearly 120 L/year/inhabitant (Kacimi El Hassani, 2013). This food occupies a preponderant place in the food ration of Algerians, it provides the largest share of proteins of animal origin. A key player in the food industry, the milk sector is experiencing annual growth of 8% in Algeria (Hamiroune *et al.*, 2014).

Milk is considered a complete and balanced food because of its richness in several nutritive elements (proteins, lipids, mineral salts, lactose and vitamins). Milk is an excellent culture medium for several microorganisms that will spoil the product.

The microbiological quality of raw milk arouses the interest of different players in the sector. From a consumer point of view, hygienic quality is of concern to consumers who are becoming more and more demanding of it. Raw milk and the products derived from it must provide health guarantees (Nanu*et al.*, 2007). Consumption of these products can pose a danger to public health (Oliver *et al.*, 2009).

The microbial composition of raw milk is often characteristic of the farm. This particularity is probably linked in part to the milking practices carried out on each farm (Monsallier *et al.*, 2012). The majority of bacterial species identified in milk originates from the environment in the milking parlor (air, food used during milking, teats) (Normand *et al.*, 2007). Poor milking technique and poor hygiene is therefore responsible for the introduction of germs into the udder and contamination of the milk. The influence of milking and the milking machine on mammary

infections of dairy cows is now well known. Since such infections are multifactorial in origin, it is always very difficult to determine with precision the share due to the milking machine itself (Mezine, 2006).

Producing quality milk and maximizing the value of milk production requires the use of a suitable milking machine, well regulated, well used and well maintained (M'sadak *et al.*, 2012).

In order to monitor the safety of food, it is imperative to determine the bacteriological quality of raw milk intended for human consumption, which prompted us to carry out this work. The main objective of the present study is the evaluation of the microbiological quality of raw milk from cows in the region of Oum El Bouaghi(Algeria) and to compare the microbiological quality between raw milk from cows obtained by the two milking methods mechanical and manual, and its risk to the health of consumers.

MATERIALS AND METHODS

2.1. Sampling

The cow's milk used is taken from two farms in the wilaya of Oum El-Bouaghi. Each farm has about 20 cows. The 10 cows were taken at random for manual and mechanical milking during the month of May 2019 at 6.30 a.m. on two days of the same week. Samples 1 to 8 (4 manual and 4 mechanical) come from a first farm and samples 9 to 20 (6 manual and 6 mechanical) from the second farm. The milk collected for microbiological analyses is collected aseptically in sterile and labeled plastic bottles. The first jets are eliminated and the bottle is filled to 2/3 of its capacity. We used the individual milk sampling technique described by Guiraud (2003). The milk samples were sent to the laboratory of food microbiology, University of Batna 1) at a temperature of 4°C, in a cooler fitted with frozen ice cubes.

2.2. Microbiological analyses

Microbiological analyses were performed at 8:30 a.m., 2 hours after collection.

From milk homogenized beforehand by rotational movements, series of decimal dilutions were carried out in a solution of sterile physiological water to facilitate enumeration until the dilution of 10^{-6}.For enumeration of salmonella, buffered peptone water was used as a diluent instead of physiological water.

* Total mesophilic aerobic flora (TMAF) was counted on PCA agar (Plate Count Agar) incubated for 72 h at 30°C.
* Coliforms were sought on VRBL agar (Violet Red Bile Lactose) and incubated for 24 hours at 37°C for total coliforms and at 44°C for fecal coliforms.
* Fecal streptococci were counted on Rothe agar after 48 hours incubation at 37°C. Each Rothe tube found positive during the presumption test was subcultured in a tube of Litsky medium and incubated at 37°C for 24 hours.
* Staphylococci were counted on Baird Parker agar and incubated for 48 hours at 37°C.

The confirmation of *Staphylococcus aureus* was carried out by the search for the coagulase (+) colonies. It was done by adding 0.5 ml of rabbit plasma to 0.5 ml of the bacterial culture. Incubation took place at 37°C for 24 hours. The formation of a coagulum indicates the presence of a coagulase.

* For salmonella, a pre-enrichment was carried out on the Selenite Cysteine (SC) broth 16 to 20 hours at 37°C, followed by an enrichment on Tetrathionate broth for 24 hours at 37°C, then the count and the isolation were carried out on Hecktoen agar after incubation for 24 hours at 37°C.
* The sulfito-reducing clostridiums were counted on the meat-liver (LM) agar medium supplemented with iron alum and sodium sulfite. To promote anaerobic conditions, the dilutions are first subjected to heating at 80°C for 10 minutes, then to immediate cooling under tap water, with the aim of eliminating the vegetative forms and keeping only the sporulated ones. From these dilutions, 1 ml of each dilution was aseptically placed in the center of a Petri dish. Then about 15 ml of ready-to-use LM agar was added. After rotary movements in the form of 8, the plates were

left to solidify on the bench and incubated, aerobically for 24 hours at 37°C.
* Yeasts and molds were counted on the Sabouraud 4% glucose medium and incubated for 5 days at 22°C.
* The lactic bacteria were counted on the Man Rogosa Sharpe(MRS) medium and incubated for 48 hours at 30°C.

2.3. Statistical analyses

The data obtained were first entered in Excel tables (Microsoft 2010) for simple descriptive statistical analysis (mean, standard deviation, maximum, minimum), then compared using the Graph Pad Prism 7 version software (7.00) using:
- A t-test (Student) for paired samples (FTAM count).
- Mann Whitney test for samples with non-normal distribution (enumeration of total coliforms, fecal coliforms, *Staphylocccus aureus* and lactic bacteria)

Normality for all samples was checked by Shapiro-Wilk test.
Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the microbiological analyses of the milks analyzed, expressed in CFU/ml are presented in Table 1. They represent the load of the different microorganisms sought in the raw milks analysed.

- **Enumeration of the total aerobic mesophilic flora**

The samples taken showed a load of microorganisms in the total flora which varied from 1×10^7 to 2.5×10^7 CFU / ml, for an average of $1.8\times10^7 \pm 0.5\times10^7$ CFU / ml for manual milking and from 0.6×10^7 to 2.6×10^7 CFU / ml, for an average of $1.44\times10^7 \pm 0.66\times10^7$ CFU / ml for mechanical milking (Table 1 and Figure 1).

Table 1: Levels of the various microorganisms counted in the 20 milk samples (CFU/ml)

Floras (CFU / ml)		Minimum	Maximum	Mean	Standard deviation	Standards (CFU/ml) (JORA, 1998)	Standards (CFU/ml) (JORA, 2017)
Total aerobic mesophilic flora (10^7)	**M**	1	2,5	1,8	0,50	10^5	3×10^6
	ME	0,6	2,6	1,44	0,66		
Total coliforms (10^4)	**M**	Absence				/	/
	ME	0	6,4	2,1	2,40		
Fecal coliforms (10^4)	**M**	Absence				10^3	5×10^3
	ME	0	3	0,69	1,06		
Fecal streptococci	**M**	Absence				Absence/0,1 ml	Absence/0,1 ml
	ME	Absence					
***Staphylococcus aureus* (10^3)**	**M**	0	2,5	0,93	0,77	Absence	10^3
	ME	Absence					
Yeasts and molds	**M**	Absence				/	/
	ME	Absence					
Lacticbacteria (10^4)	**M**	0	4	0,7	1,33	/	/
	ME	0	11	5,12	4,07		
Clostridium	**M**	Absence				Absence	/
	ME	Absence					
Salmonella	**M**	Absence				/	Absence/25 ml
	ME	Absence					

M: Manual Milking.**ME:**Mechanical milking.

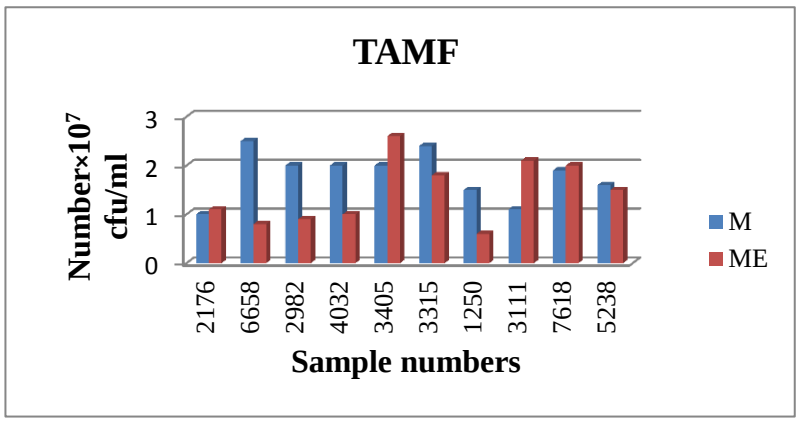

Figure 1: TAMFs of the raw milk samples obtained by two milking methods (M: manual milking, ME: mechanical milking)

The total aerobic mesophilic flora is a good indicator of the global contamination and provides information on the hygienic quality of raw milk (Guinot-Thoms *et al.*, 1995). It is the most sought-after flora in microbiological analyses.

The results obtained for TAMF of the two milking methods always were above the limits announced by JORA (2017) (3×10^6CFU/ml) and by JORA (1998) (10^5 CFU/ml). They were also higher than the two French and American regulations which(5.10^5 CFU/ml and 3×10^5 CFU/ml, respectively) (Alais, 1984).

Our results are in agreement with those of Mansour (2015), Benhedane-Bachtarzi (2012), Labioui *et al.*, (2009), Afif *et al.*, (2008), and are above the limits announced by Kaouche-Adjlane and Mati (2017) 21×10^3 CFU/ml, Belarbi (2015) 1.3×10^4 CFU/ml and Hamiroune (2014) 8.3×10^5 CFU/ml.

The high levels of total flora can be interpreted as an indication of poor hygienic practice during milking.

The statistical analysis indicated that the TAMF counts were not significantly different between manual milking and mechanical milking ($P = 0.2088$). However, the results showed a very significant difference ($p < 0.001$) for the TAMF count between manual cow milking and the standards of JORA, 2017 and mechanical milking and the standards of JORA, 2017, $p < 0001$ and $p = 0.0004$, respectively.

- **Enumeration of total coliforms**

The samples showed an absence of total coliforms for manual milking; and a load of total coliforms which varied from 0 to 6.4×10^4 CFU/ml, for an average of $2.1\times10^4 \pm 2.40\times10^4$CFU/ml for mechanical milking (table 1). The results of the enumeration of the total coliforms of the different raw milk samples are shown in figure 2.

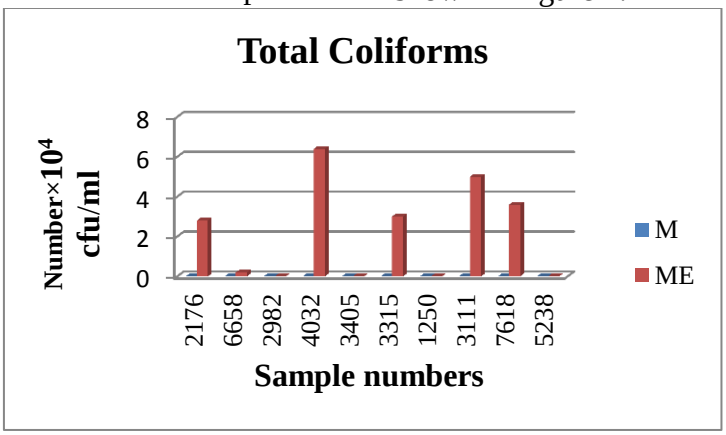

Figure 2: Total coliforms of raw milk samples obtained by two milking methods (M: manual milking, ME: mechanical milking)

The presence of coliforms is not necessarily a direct indication of fecalcontamination. Some coliforms are, in fact, present in the wet residues encountered in dairy equipment (Larpent, 1990).This finding might explain the presence of total coliforms in the samples of raw milk obtained by mechanical milking and their absence in those obtained by manual milking.

Our results are lower than the counts found by Ouinine *et al.* (2004) 1.07×10^{7} CFU/ml. However, they are higher than those reported by Afif *et al.* (2008) 3.2×10^{5} CFU/ml.

Statistical analysis of our results shows a significant difference ($p < 0.05$) for the count of total coliforms between manual milking of cows and mechanical milking ($p = 0.0108$).

- **Enumeration of fecal coliforms**

The samples taken show an absence of fecal coliforms for manual milking; and a load of fecal coliforms which varies from 0 to 3×10^{4} CFU/ml, for an average of $0.69\times10^{4} \pm 1.06\times10^{4}$ CFU/ml for mechanical milking (table 1).

The results of the enumeration of fecal coliforms from the different raw milk samples are shown in (figure 3).

The Algerian standard for fecal coliforms is fixed at 5.10^{3} CFU/ml (JORA, 2017). We found that our results were very variable and superior to this standard.

The search for microorganisms indicative of contamination of faecal origin makes it possible tojudge the hygienic state of a product. Even at low levels, they would show degraded hygienic conditions during milking or during transport (Labioui*et al.*, 2009).

Tormo *et al.*, (2006) cited by Cauquil (2011) showed that the milking machine provided more coliforms than manual milking. This is due to its cleaning and adjustment (possibility of milk reflux) which explains the presence of fecal coliforms in raw milk samples obtained by mechanical milking and their absence in those obtained by manual milking. So, it is necessary to increase the frequencies of washing of the milking machine: this is done only once a day, the evening milking being followed by a simple rinsing with water (Michel *et al.*, 2006).

Our results were higher than those reported by Ghazi and Niar (2011) (1.7×10 CFU/ml) and similar to those obtained by Mansour (2015) (2.6×10^{4} CFU/ml), Hamiroune *et al.* (2014) (4.6×10^{4} CFU/ml) and Afif *et al.* (2008) (32×10^{4} CFU/ml), but are significantly lower than the results reported by Ouinine *et al.* (2004) (1.99×10^{6} CFU/ml).

The statistical analysis showed a significant difference ($p < 0.05$) for the counting of fecal coliforms between manual milking of cows and mechanical milking ($p = 0.0325$).However, the difference was not significant ($p > 0.05$) for the counting of fecal coliforms between mechanical milking and the standards of JORA, 2017 ($p = 0.585$).

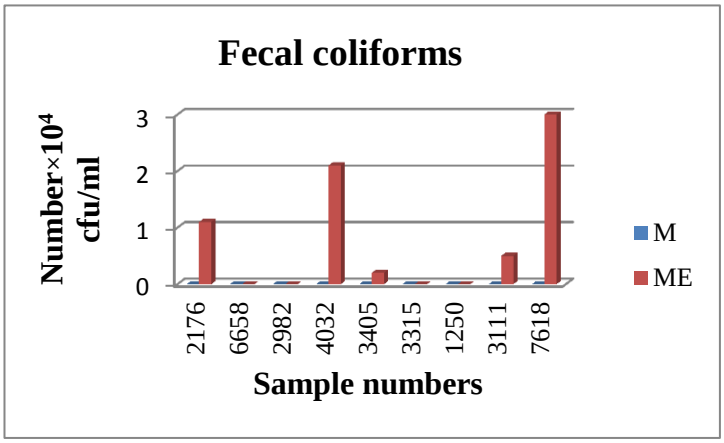

Figure 3: Fecal coliforms of raw milk samples obtained by two milking methods (M: manual milking, ME: mechanical milking)

The results showed a total absence of fecal streptococci for both types of milking (Table 1). The Algerian standard JORA (2017) for fecal streptococci is the absence of the germ in 0.1 ml of raw milk. Therefore, our results are in accordance with the Algerian standard.

The rate of streptococci is related to the state of health of the cows, the hygienic conditions of the milking, and the possible contaminations during the enumeration (Labioui *et al.*, 2009). The total absence of fecal streptococci in all samples could be explained by the good state of health of the cows.

Our results are significantly lower than those reported by Benhedane-Bachtarzi (2012) (55.4×10^4 CFU/ml), Labioui*et al.* (2009) (0.4×10^3UFC/ml) and Afif*et al.* (2008) (10^2 CFU/ml).

- **Enumeration of *Staphylococcus aureus***

The load of *Staphylococcus aureus* which varied from 0 to 2.5×10^3 CFU/ml, for an average of $0.93\times10^3\pm0.77\times10^3$ CFU/ml for manual milking; and its absence for mechanical milking (table 1).

The results of the enumeration of *Staphylococcus aureus* from the different raw milk samples are shown in (Figure 4).

Our results showed the absence of *Staphylococcus aureus* in milk obtained by mechanical milking. These results complied with the standard of JORA (1998) (Absence). Whereas the milk obtained by manual milking, presented a contamination with an average of $0.93\times10^3 \pm 0.77\times10^3$ CFU/ml. These results are higher than the JORA (2017) standard (10^3UFC/ml).

The main sources of contamination are, first of all, the udder. Staphylococcal mammary infections represent the main source of contamination of milk during production (Thieulon, 2005).

Our results are in agreement with those of Hamiroune *et al.* (2014) (0.9×10^3 CFU/ml), and close to the results obtained by Aggad *et al.* (2009) (3.5×10^3 CFU/ml). However,they were lower than those obtained by Afif*et al.* (2008) (8.0×10^4 CFU/ml) and Mennane *et al.* (2007) (1.2×10^6 CFU/ml).

The high levels of total flora can be interpreted as an indication of poor hygienic practice during milking.

The results showed a very significant difference ($p < 0.001$) for the counting of *Staphylocccus aureus* between manual milking of cows and mechanical milking ($p = 0.0007$) However, the results obtained show a significant difference ($P < 0.01$) for the enumeration of *Staphylocccus aureus* between manual milking of cows and the JORA standard, 2017 ($p = 0.0043$).

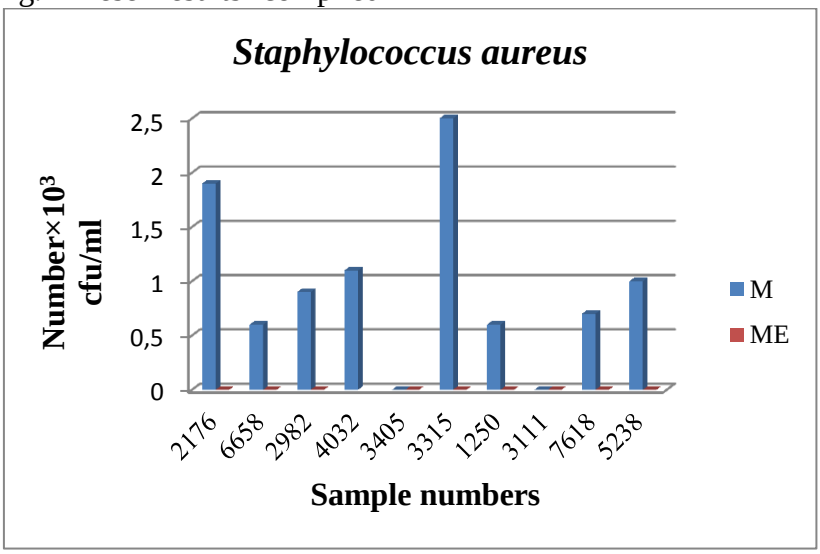

Figure 4:*Staphylococcus aureus* of raw milk samples obtained by two milking methods (M: manual milking, ME: mechanical milking)

- **Enumeration of yeasts and molds**

The resultsshowes a total absence of yeasts and molds in milk obtained by the two types of milking methods (table 1).

According to Snappe (2010), yeasts and molds cause manufacturing accidents, deterioration of taste, swelling, poor presentation and a reduction in the shelf life of milk products.

The total absence of yeasts and molds in all the samples could be explained by the season of the sampling. In fact, the samples were taken during the month of May. Indeed, many authors have reported an increase in the number of yeasts and molds in ensiled feeds used mainly in the winter season. These microorganisms are very often transferred from animal foodstuffs to milk (Lopez *et al.*, 2003; Tasci, 2011). Thus, the bacteriological quality of milk could be associated both with the nature of winter fodder (silage and hay) and with milking hygiene (Agabriel, 1995).

Our results are significantly lower than those reported by Kaouche-Adjlane and Mati (2017), who found an average of 20×10^3 CFU/ml for yeasts and an average of 1×10^2 CFU/ml for molds.

- **Enumeration of lactic bacteria**

The results showed a load of lactic bacteria which varied from 0 to 4×10^4 CFU/ml, with an average of $0.7\times10^4\pm1.33\times10^4$ CFU/ml for manual milking; and from 0 to 11×10^4 CFU/ml with an average of $5.12\times10^4 \pm 4.07\times10^4$ CFU/ml for mechanical milking (table 1).

The results of the enumeration of lactic bacteria from the different raw milk samples are shown in figure 5.

Lactic bacteria belong to a group of beneficial bacteria, whose virtues are similar, and which produce lactic acid as the final product of the fermentation process (Prescott *et al.*, 2010). Lactic bacteria can be considered probiotics. Their ability to develop at low pH and simultaneously produce active substances (lactic acid, acetic acid, hydrogen peroxide and bacteriocin, etc.) explains their bacteriostatic or bactericidal role towards harmful species responsible for the sensory defects of fermented foods or with public health risks (Siboukeur, 2007).

The results obtained are lower than those found by Labioui *et al.* (2009) (8.32×10^5 CFU/ml).

The results showed a significant difference ($P < 0.01$) for the enumeration of lactic acid bacteria between manuals and mechanical milking ($P = 0.0058$).

- **Enumeration of *Clostridium***

The results showed a total absence of *Clostridium* for the two types of milking. (Table 1).

The standard for clostridium is the absence of the germ in raw milk. The results obtained complied with the standard recommended by JORA (1998) (Absence), and the results of Mansour (2015) and Labioui *et al.* (2009). So, these results showed good hygiene conduct at the time of sampling.

Clostridiums are therefore capable of surviving in the environment and of contaminating any type of food or material if hygiene and sterilization conditions are not respected (Lebres, 2002).

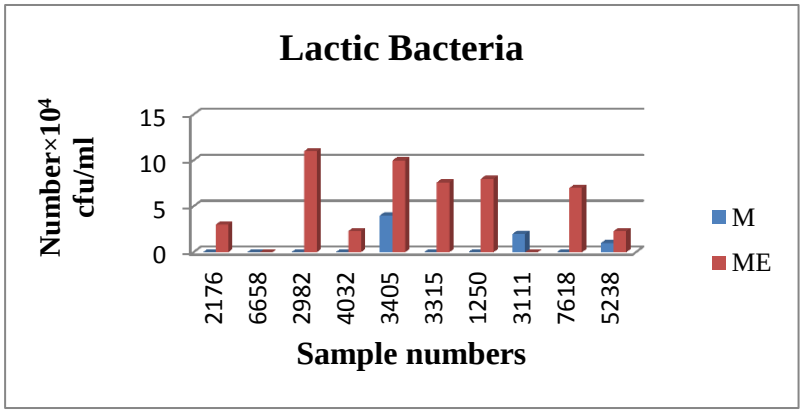

Figure 5: Lactic bacteria of raw milk samples obtained by two milking methods (M: manual milking, ME: mechanical milking)

- **Enumeration of Salmonella**

The results showed a total absence of Salmonella for both types of milking.(table 1). The Algerian standard JORA (2017) for Salmonella is the absence of the germ in 25 ml of raw milk. Our results are in accordance with the Algerian standard.

The main source of contamination of raw milk is the fecal excretion of salmonella, dissemination of the bacteria in the environment, then contamination of the udder skin and milking equipment and finally passage into milk (Guy, 2006).

Our results are in agreement with those found by Mansour (2015), Labioui *et al.* (2009) and Afif *et al.* (2008).

CONCLUSION

Microbiological analyses of the milk samples showed the complete absence of salmonella, Clostridium, yeasts, molds and fecal streptococci in milk obtainedby bothmechanical and manual methods. The absence of salmonella, streptococci and Clostridium indicates the good health status of the cows in the two farms and their good milking hygiene.

Raw cow's milk obtained by manual milking has a relatively good microbiological quality and is acceptable from a hygienic point of view.

The traditional milking of the two farms makes it possible to obtain raw milk of good microbiological quality. On the other hand, the microbiological quality of mechanically processed raw milk is relatively unsatisfactory.

In order to improve themicrobiological quality of raw milk obtained by the milking machine, different hygiene measures must be applied by farmers where the teat cups of the milking machine must be cleaned and disinfected before and after each milking time, the first milk jets must be discarded before milking and a mixture of water and cleaning product solution must be used instead water alone for rinsing the milking machine (method practiced by the majority of the farmers).

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