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Antibiotics Residues, *Staphylococcus aureus,* Total and Fecal Coliforms in Pasteurized Reconstituted Cow Milk in the Algerian East

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Abstract: The present study was carried out to verify the presence of antibiotics residues *Staphylococcus aureus* (S. *aureus*), Total Coliforms (TC) and Fecal Coliforms (FC) in pasteurized reconstituted partially skimmed and packaged cow milk sold and consumed in the Eastern of Algeria. To realize this work a total of forty four pasteurized reconstituted cow milk samples were collected, from retail space. Antibiotics were screened using the Delvotest and the Beta star Combo test (BSCT). TC and FC were enumerated in VRBL agar (Violet Red Bile Lactose), while the S. *aureus* was isolated in Baird Parker agar supplemented with Rabbit Plasma Fibrinogen [RPF]. Out of 44 samples of reconstituted milk studied, 43.18, 27.27 and 43.18% showed contamination by S. *aureus*, FC and TC respectively. Antibiotics residues using Delvotest were detected in 04.55% of samples. Using the BSCT, 4.55% of samples were positives for Beta-lactams residues. The presence of likes these pathogenic microorganism and the antibiotics residues indicates a potential health hazard to those who consume milk from this region.

Key words: Pasteurized Milk · *Staphylococcus aureus* · Total Coliforms · Fecal Coliforms · Antibiotics Residues · Algerian East

INTRODUCTION

The Food and Agriculture Organization (FAO) recommend a "safe" protein consumption of 58 g per person per day [1]. In order to fill the deficit in proteins which have animal's origin, the Algerian population with weak income had tendency to resort generally for the consumption of reconstituted pasteurized partially skimmed and packaged milk because on the one hand as a very rich food in nutrients [2] the milk can make up for others costly products like meat for example and on the other hand, it was subsidized by the government and like this pasteurized milk presents a little public health hazard. Sure enough 1g of proteins from milk cost plus of 8 times less expensive than the same quantity from the meat. These reasons make for milk an attractive product for the Algerian household. With a mean consumption of 110 L of milk by habitant and by year, estimated 115 L in 2010, Algeria is the very important consumer of milk in the Maghreb [3]. The milk constituted so a basis product in the Algerian consumption model. This food can nevertheless represent if its hygienic quality does not controlled a danger for human health after consumption especially if it doesn't inadequately pasteurized and it may contain microorganisms of special importance to man which its presence or absence in pasteurized milk may reflects success or failure of Good Manufacturing Practices (GMP) or cause infection when consumed together with food.

In Algeria, there is a rarity in data on the hygienic quality and occurrence of antibiotics residues especially Beta-lactams and Tetracyclines in reconstituted pasteurized cow milk and there is now no national antibiotic residue monitoring program. So through this work, we want to construct a report regarding that which was throwing at first.

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MATERIALS AND METHODS

From January 2014 to April 2015 a total of forty four reconstituted pasteurized partially skimmed cow milk samples were collected, from retail space. All the samples were collected aseptically and processed immediately as per the standard protocols. All the samples were kept in the icebox, transported to the laboratory under chilled conditions and processed for microbiological and antimicrobial residues analysis. The time between sampling and transportation to the processing unit was also assessed. In order to enumerate FC and TC each sample was diluted before plating. The dilutions were made in sterilized salted peptone water. One ml of milk from each sample was poured into 9 m1 of sterilized salted peptone water in a test tube to get a dilution of [1:10]. From this, several dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared. Diluted samples were mixed thoroughly. All Petri plates were labelled with dilution factor and sample number. Coliforms were enumerated on solid medium through the dish technique on VRBL agar described by Joffin and Joffin [4]. Thus 1 ml of each of the dilutions was taken aseptically in a labelled sterile Petri dish. After we pour approximately 12 ml of molten media (Dissolved at 100 °C and then cooled down at 45 ± 1 °C) into each inoculated Petri dishes. After pouring the media, Petri dishes were shaken clockwise and anticlockwise to obtain sufficiently spaced colonies. The dishes were leaved to solidify and once they are solidified, they were poured at new a protection layer of about 4 ml against the different contaminations. This preparation was carried out in double for each dilution. The first series of dishes was incubated at 37 °C during 24 à 48 Hours and it was reserved to count the TC. The second series of dishes was incubated in water bath at 44°C during 24 à 48 Hours and it was reserved to enumerate the FC. Coliforms appear in mass in the form of small fluorescents colonies, of dark red color (Bacteria lactose +) and at least 0.5mm of diameter. S. *aureus* was researched on the solid medium of Baird-Parker agar complemented with RPF supplement. After we had dissolved 90 ml of Baird parker agar at 100°C and it had cooled down at 45°C ± 1°C; 10ml of sterile solution of RPF supplement recently prepared were added. Using a sterile spreader; we spread out 0.1ml of the inoculums in the form of three fractions on the entire medium surface (15 to 18ml of Baird Parker supplemented with RPF) solidified in the dish. The incubation was done at 37°C during 24 to 48 Hours. After growth [4], *Staphylococcus* colonies were counted and classified as for S. *aureus* if they are gray or black colonies surrounded by an opaque halo of fibrin that is clear cut, stable and well visible. Antibiotics residues were screened firstly using the Delvotest SP (DSM Food Specialties, NL) which combine the principle of agar diffusion tests with a color change of the bromo-cresol purple indicator resulting from the active metabolism of the testing microorganism in the absence of inhibitor. The milk sample is batched into micro-titration plates with pits filled the agar nutrient containing bacillus *stearothermophilus var. calidolactis.* The incubation (64 ± 1°C/2.5-3hours), at which the tested strain growing, causes that the color of bromocresol purple will change from blue violet to yellow. If the sample contains inhibitors substances, the color of the indicator will remain as it was and secondly using the Beta-star Combo test (Neogen Corporation, USA), which is a competitive receptor test in dipstick format, employs binding reagents linked to gold particles for the rapid detection assay at levels well below the Maximum Residue Limit (MRL) of both βeta-lactams and Tetracyclines antibiotics.

All statistical analyses were performed using Microsoft office Excel (2007) and IBM SPSS (Statistical Package for Social Sciences) version 20.

RESULTS

As shown in table 1, of all samples 43.18, 27.27 and 43.18% showed contamination by S. *aureus*, FC and TC respectively. Using Delvotest, antibiotics residues were detected in 27.27% of samples [2 positives and 10 doubtful samples]. Using the BSCT, 4.55% of samples were positives for Beta- lactams residues (Table 2).

Table 1: Results of microbiological analysis.

Bacteria	Prevalence [%]	Min	Max	Mean (CFU)	SD
TC	43.18	00	240000.00	14680.8333	41111.80063
FC	27.27	00	20233.33	1259.5455	3740.05357
S. *aureus*	43.18	00	2500	105.45	386.90

Min: Minimum, Max: Maximum, CFU: Colony Forming Unit.

Table 2: Antibiotics residues in reconstituted pasteurized cow milk in the Algerian East.

Used test	Results	Percentage [%]
Delvotest	Positives samples	04.55
	Doubtful samples	22.72
	Negatives samples	72.73
Beta star Combo test	Samples with Beta-lactams residues	04.55
	Samples with Tetracyclines residues	0.0
	Samples with both residues	0.0
	Negatives samples	95.45

DISCUSSIONS

The experimentation results give a very good overview of the actual situation of reconstituted pasteurized cow milk quality in the dairy sector. A wide variability was observed in the content of the researched Coliforms bacteria (Big SD). The average content of TC and FC was 1.47×10^4 CFU/ml and 1.3×10^3 CFU/ml respectively, which were extremely high than the standard threshold set by Algerian regulations which recommend less than 10 colonies/ml for TC and the absence for FC [5]. Coliforms are considered as 'indicator organisms' because their presence in food indicates some form of contamination [6]. Coliform groups are most widely applied in the food industry as sanitation and process integrity indicators and for Hazard Analysis Critical Control Point (HACCP) verification [7]. Pasteurized milk shouldn't contain any coliform bacteria as though the later can't survive the pasteurization temperature [8, 9]. Their presence in pasteurized milk indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipe lines [8]. Our results in TC stay good if it compared with the results founded for example in Brazil by de-Oliveira *et al*. [10] who reported a TC load of 5.53×10^{10} CFU/ml, contrary they stay very bad than to that reported in 2012 in Bangladesh by Saha et Ara [6] (13.12 CFU/ml) and also very bad to that recorded by Aggad *et al*. [11] in the west of Algeria which found a load of 3.3 ± 0.58 CFU/ml.

The research of indicator microorganisms of a fecal contamination allows to evaluate the hygienic quality of a product. The presence of FC is often linked with the presence of pathogenic enterobacteria like *Salmonella*, *Shigella*, *Yersinia* and some biotypes of *E. coli* [12]. Moreover, the presence of FC usually indicates recent fecal contamination, because these bacteria cannot survive apart from the intestine for a long time and their number is generally proportional to pollution degree produced by feces. Aggad *et al.* [11] reported in 2010, a FC average of 24 ± 47 CFU/ml. In Brazil, De-Oliveira *et al.* [10] found a FC load of 7.16×10^8 CFU/ml, clearly higher than the result reported in this work.

The milk is an excellent medium for the growth of a wide number of microorganisms including S. *aureus* [13, 14]. The research of *S. aureus* permits to provide if a food presents a hazard for the consumer because they can produce an enterotoxin cause of alimentary intoxications. S. *aureus* in milk is one of the most common causes of reported foodborne diseases, which is a major risk to public health [15]. The results in S. *aureus* are also largely variable (Big SD). With a mean load of 1.05×10^2 CFU/ml, 43.18% of analyzed samples contained S. *aureus* and this is above the Algerian standard which limits acceptability threshold to 1 CFU/ml [5]. Pasteurized milk is very favorable for the growth of S. *aureus* than the raw milk, because this microorganism is a poor competitor in the presence of others bacterial floras [16]. *S. aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents [17] thus their presence in pasteurized milk is an indication of poor sanitation or post pasteurization contamination. In the western of Algeria, Aggad *et al*. [11] studied in 2010 the presence of this germ in 54 pasteurized cow milk and 46 reconstituted pasteurized cow milk and showed that the prevalence was 37 and 21.74% with an average load of $5.9 \times 10 \pm 9.7$/ml and $6.7 \times 10 \pm 10.67$ CFU/ml respectively. Also, in the west of Algeria, Kabir and Niar [18] showed through their study the absence of *S. aureus* in the 35 analyzed samples. Fernane *et al*. [15] detect S. *aureus* in 61% of the checked milks. Contamination in milk by S. *aureus* was detected in other countries, with similar results as the study in question. In Bahia of Brazil, de-Oliveira *et al*. [19] reported a count in S. *aureus* of 3.5×10^3 CFU/mL, with a prevalence of 30% among the 20 pasteurized milk samples. In Iran, Mirzaei *et al.* [20] and Vahedi *et al*. [21] found the same prevalence (2%).

The prevention of antibiotic residues is an important aspect of milk quality. The milk must be exempt from antibiotics and all others drug residues [22]. All antibiotics can be detected in milk using the Delvotest kit. Also all of βeta-lactams and Tetracyclines can be detected in milk using the Beta star Combo kit. Among 44 analyzed samples, 27.27% contained antibiotics residues (positives and doubtful samples); which is a high percent for reconstituted pasteurized cow milk in Algerian's East region. This percentage translates a lack of an antibiotics residues screening in the imported milk powder. We also note throw this work that the positives samples for Delvotest were the same for BSCT. While they which are doubtful for Delvotest [22.72%] were negatives for BSCT.

Antibiotics residues are dangerous for consumers and result in serious problems during processing of the milk [23]. They can induce a number of potential problems for human health like damage of brain especially by Chloramphenicol [23] perturbations of normal intestinal flora, digestives troubles & allergic reactions [24, 25, 26] and a decreased antimicrobial susceptibility in bacteria of medical importance [27]. Other hazards for the health is abetting the cultivation of antibiotic resist microorganisms

[23, 26] and they can masque or cover up the presence of pathogenesis bacteria [4]. Also problems during processing of milk which can occur during production of fermented milk products, caused by inhibition of the wished microorganisms such as added cultures [23]. For all these reasons, the law forbids the sale of milk containing antibiotics.

In Montenegro, it was studied using the "Delvotest®Accelerator" for the presence of inhibitory substances in raw milk and it was reported that from 6161 tested samples, 7.84 % were positive [25]. Fonseca *et al*. [28] observed the presence of antibiotics residues in Brazilian UHT milk, relating their presence in 4% of the samples. In morocco there is a rarity of antibiotics residues investigations in milk, Sraïri *et al*. [29] found that from 60 samples, 25 % were positive. In Coast Ivory, Kouamé-Sina *et al*. [22] showed that 24.7% of examined samples were positives for antibiotics residues. In Iran, Movassagh and Karam studied the prevalence of antibiotics residues in 100 cow raw milk samples of collection using the Copan test and showed that of all samples 5% were positives [30]. In Egypt, the incidence of antibiotic residues in raw milk samples was 23.6% and 20% by using Delvotest® SP NT and Betastar® combo HS kits, respectively [31].

CONCLUSIONS

The dairy situation is alarming. The study result indicates clearly the lack of what we called GMP (Good Manufacturing Practices) in industries that manufactured reconstituted milk. The presence of antibiotics residues, pathogenic and indicator organisms such as S. *aureus* and Coliforms may lead to a hazard against public health. Therefore practice and regulations, such as on-site pasteurization and implementation of HACCP following established standards, should be introduced to facilitate the manufacture of reconstituted pasteurized packaged cow milk of high hygienic quality.

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