

Examination of the behaviour of bacterial pathogens in raw milk

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Among recent trends towards less processed foods is a growing consumer demand for raw (unpasteurised) dairy products. In spite of the public health risks and an increased awareness of pathogens in milk, proponents of raw milk consumption are of the view that pasteurisation kills beneficial microorganisms believed to have antimicrobial properties towards pathogens. This study thus aimed to examine the behaviour of bacterial pathogens in raw milk. Four pathogenic bacteria relevant to the dairy industry, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus*, were investigated. Milk-spiking experiments were performed at different temperatures over 5 days in raw milk collected from different sources. It was observed that the introduced pathogens showed limited growth at a low storage temperature (refrigeration); however, increased growth rates were observed at higher temperatures of 8°C and 15°C. In no cases were the pathogens eliminated. These observations suggest that consumption of raw milk is potentially hazardous, especially if the milk is subjected to mild and moderate temperature abuse, since bacterial pathogens remain viable, and indeed multiply, in the presence of normal milk microbiota.

Keywords raw milk; foodborne illness; food safety; public health

1. Introduction

Milk is a source of food that is widely available, generally in plentiful supply, inexpensive and, for the most part, safe. Two main factors contribute to the microbiological quality of milk: (a) the existence of organisms in secreted milk (pre-harvest) and (b) the contamination of milk at the time of collection, processing, packaging, transport, distribution and storage (post-harvest). If pathogenic bacteria are among the contaminants, the product will pose a food safety threat to consumers [1]. However, advances in the dairy industry over the last few decades (animal production and herd maintenance, food processing and hygiene, proper storage facilities and refrigeration) has reduced, if not eliminated, several foodborne diseases that were major concerns to humans in the past century. Although milk was once considered to be an important vehicle for foodborne diseases, processes such as pasteurisation and ultra-high-temperature (UHT) treatments have and are being used to produce safe and clean milk [1]. Strict food safety standards, law enforcement and regulations, routine inspections and quality testing have minimised milkborne disease outbreaks [2].

In recent years, however, in many developed countries, there have been changes in lifestyle trends and consumer shifts towards more organic and fresh products. There is now a growing consumer demand for raw (unpasteurised) dairy products [3]. Raw milk advocates are of the opinion that the processes of pasteurisation and homogenisation have some serious and detrimental effects on the quality of milk [1]. Proponents of raw milk are of the view that raw milk is a living fresh food, whereas, the process of pasteurisation kills off many of the beneficial components [4] including beneficial microorganisms which have antimicrobial properties towards pathogens [1]. However, scientific evidence to substantiate these claims is lacking.

The “raw milk revolution” is becoming a global issue of concern to health and regulatory authorities [5]. There exists an abundance of information on the potential risks involved in consuming raw milk. Raw milk harbours a wide array of pathogenic microorganisms [6,7], many of which have been responsible for outbreaks of foodborne illness locally and globally [8]. Pathogens associated with human illness linked to the consumption of raw milk and raw-milk products include enterohaemorrhagic *Escherichia coli* (EHEC), *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*. These organisms have been known to cause a range of foodborne illnesses such as gastroenteritis and diarrhoea but may also cause a number of other types of severe illnesses such as meningitis, septicaemia, neurological conditions and haemolytic uraemic syndrome (HUS) [9].

Most legislative bodies in developed countries have strict food practices for the production and sale of milk in order make it safe for human consumption [8]. Australia has a set of guidelines laid down by Food Standards Australia New Zealand (FSANZ) which are controlled and followed by the relevant authorities in each state. Sale of raw bovine milk for consumption is strictly prohibited; however, the sale of raw dairy products packaged and advertised for cosmetic reasons occurs in many jurisdictions [8]. Therefore, since raw milk cannot be legally sold, the same set of laws that apply to the production and sale of pasteurised milk may not necessarily apply. While an abundance of published and unpublished literature exists internationally showing that raw cow’s milk is often contaminated with pathogenic bacteria, such data from Australian sources are scarce. Hence, this study was designed to determine the overall quality of raw milk samples obtained from a local dairy farm, from milk tankers at local dairy processing site and commercial “cosmetic” bath milk sold at an organic and health food retail outlet and to examine the behaviour of pathogens in raw milk in the presence of the normal milk microbiota.

2. Materials and Methods

2.1. Raw milk samples

Raw milk samples were sourced from a dairy farm situated in Gippsland, Victoria (about 2 hours driving distance from the laboratory), from milk tankers at a local dairy processing plant (about 0.5 hours) and commercial “cosmetic” raw milk sold at organic and health food retail outlet situated approximately 10 minutes’ walk from the laboratory. All samples were transported directly to the laboratory and refrigerated until use.

2.2. Examination of microbiological quality of raw milk

A standard aerobic plate count (APC) was performed using the surface spread and pour plate methods for all raw milk samples. Samples were also examined for the presence of specific microbes using standard microbiological selective media: Chromocult Coliform Agar (Merck) for detection of *Escherichia coli*, other coliforms and non-coliforms; Sorbitol MacConkey Agar (SMAC) (Difco) for detection of *E. coli* O157:H7; Mannitol Salt Agar (MSA) (Difco) for detection of *Staphylococcus aureus*; Xylose Lysine Deoxycholate Agar (XLD) (Difco) for detection of *Salmonella* spp.; and Potato Dextrose Agar (PDA) (Difco) for detection of yeasts and moulds. For the detection of *Salmonella* spp., non-selective pre-enrichment in buffered peptone was performed. Milk samples (10 mL) were added to 90 mL of 1% peptone water and incubated at 37°C for 24 hours prior to plating XLD agar. All milk samples were tested in triplicate.

2.3. Milk-spiking experiments

For the milk-spiking experiments, streptomycin-resistant derivatives of four pathogenic bacteria, *Escherichia coli* O157:H7 (EDL 933) (ATCC 700927), *Listeria monocytogenes* serotype 4b (ACM 98), *Salmonella enterica* (ATCC 13311) and *Staphylococcus aureus* (ATCC 12600), were used. These bacteria were prepared by growing the parent strains on Brain Heart Infusion (BHI) agar supplemented with 300µg/mL streptomycin and selecting spontaneously arising streptomycin-resistant mutants. These were grown until pure cultures were derived. A single colony from an overnight culture of each antibiotic-resistant bacterium was transferred into 3 mL of BHI broth (Oxoid) and incubated for 24 hours at 37°C. After incubation, 0.1 mL of the culture was added to 9.9 mL of peptone water (Oxoid) and mixed thoroughly by vortexing. A 10-fold serial dilution was performed and the 10⁻⁷ dilution was then spiked into triplicate milk samples. This resulted in an initial inoculum of 10²-10³ cfu/mL. For each milk sample collected, spiking experiments were performed within 30 minutes of the milk samples reaching the laboratory. Milk samples were spiked with each bacterium in three sets in order to facilitate their incubation at temperatures of 5°C (the desired or legally required storage temperature), 8° C which represents “real world” mild temperature abuse [10] and 15° C which represents exposure of the milk to higher temperature abuse. Viable counts of all samples were performed each day for 5 days. Serial dilutions were plated onto BHI agar supplemented with 300µg/mL streptomycin to determine the number of streptomycin-resistant bacteria in each sample.

3. Results

3.1. Microbiological quality of raw milk samples

Seventeen raw milk samples were collected as part of this study. These included two samples collected immediately after milking of dairy cows at a local dairy farm on two different occasions, 10 samples from milk tankers at a local dairy processing plant and five “cosmetic” raw milks. The microbiological characteristics are given in Table 1. There was considerable variation observed in the numbers and types of microorganisms detected. Overall, the cosmetic milks were of poorest microbiological quality. This is likely to be the result of the longer time between collection and testing for the commercially prepared and processed cosmetic milk compared to the freshly collected farm and tanker milks. None of the samples showed the presence of *Salmonella* or *E. coli* O157:H7. All samples were stored at refrigeration temperatures and the APC determined after 5 days. In all cases, the total viable counts increased by approximately 1-2 log₁₀ cfu/mL, although the pH of the milk did not show considerable variation (<0.1 pH unit).

Table 1 Microbiological quality of raw milks used in this study

Milk type	Sample	Microbiological parameter*					
		APC**	<i>E. coli</i>	Other coliforms	Non-coliforms	<i>S. aureus</i>	Yeasts and moulds
Cosmetic	C1	1.97	0	0	0	0	0.69
	C2	3.43	0	1.30	2.07	2.67	2.77
	C3	4.77	0	0.69	1.17	1.54	2.65
	C4	4.68	0.69	1.17	0.97	1.03	0.71
	C5	4.14	0	0	2.37	2.31	1.69
Tanker	T1	3.19	1.54	1.69	1.74	2.87	2.27
	T2	3.91	0.69	0	0.69	2.04	2.23
	T3	3.41	1.47	0.69	0	2.27	2.25
	T4	3.69	1.00	1.54	1.97	2.46	2.54
	T5	2.88	1.47	2.57	1.77	2.39	3.01
	T6	3.47	1.00	1.17	0.69	2.04	1.84
	T7	2.86	0.69	1.30	1.69	2.26	2.25
	T8	3.43	1.30	2.32	1.39	2.07	2.59
	T9	2.67	1.00	0.69	1.30	2.04	1.47
	T10	2.24	1.65	1.00	2.04	2.32	2.14
Farm	F1	2.96	2.21	1.60	1.47	2.19	2.67
	F2	2.65	2.39	1.62	1.50	2.15	2.81

*All data are presented as log₁₀ cfu/mL.

**APC = aerobic plate count

Bolded samples were used in spiking experiments.

3.2. Growth behaviour of pathogenic bacteria in raw milk

The main focus of this study was to examine the growth behaviour of bacteria in raw milk at different temperatures. Selected milks (samples C4, T9 and F1) were inoculated individually with four pathogenic bacteria and incubated at 5°C, 8°C or 15°C. The results are presented in Figures 1-4. The viable counts in the cosmetic milk (C4) showed an initial decline for most of the bacteria and this decline was particularly noticeable at the lowest temperature. The cosmetic milk had the highest APC of the three samples used in spiking experiments and this initial decline may be explained by competition from the resident microbiota. Nonetheless, with further incubation, the viable counts of streptomycin-resistant bacteria recovered and often exceeded the initial inoculum levels, indicating that the pathogens were able to compete with the resident microbiota and grow in the milk environment.

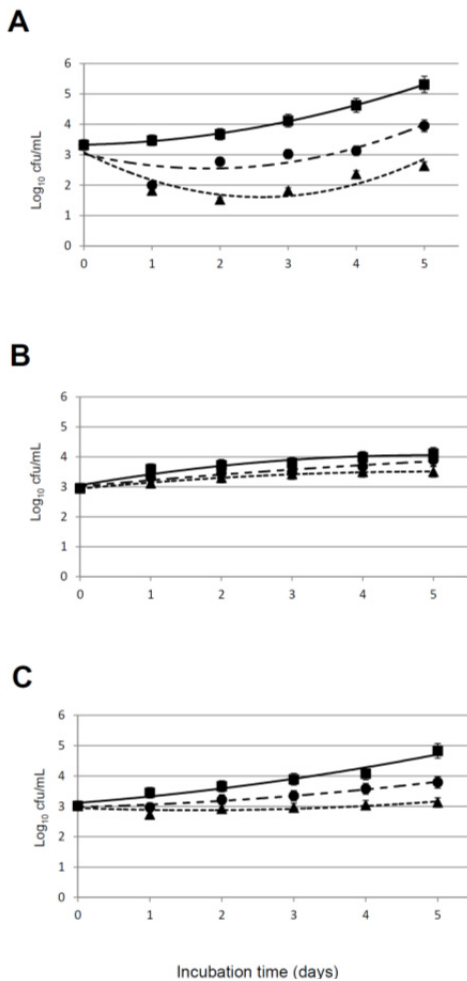


Fig. 1 Growth patterns of *E. coli* O157:H7 in raw cosmetic milk (A), tanker milk (B) and farm milk (C). For each graph, results are for incubations performed at 15°C, 8°C and 5°C are represented by squares (■), circles (●) and triangles (▲), respectively. Solid and dashed lines indicate trendlines. All data points indicate the average of triplicate viable counts and error bars indicate standard deviations.

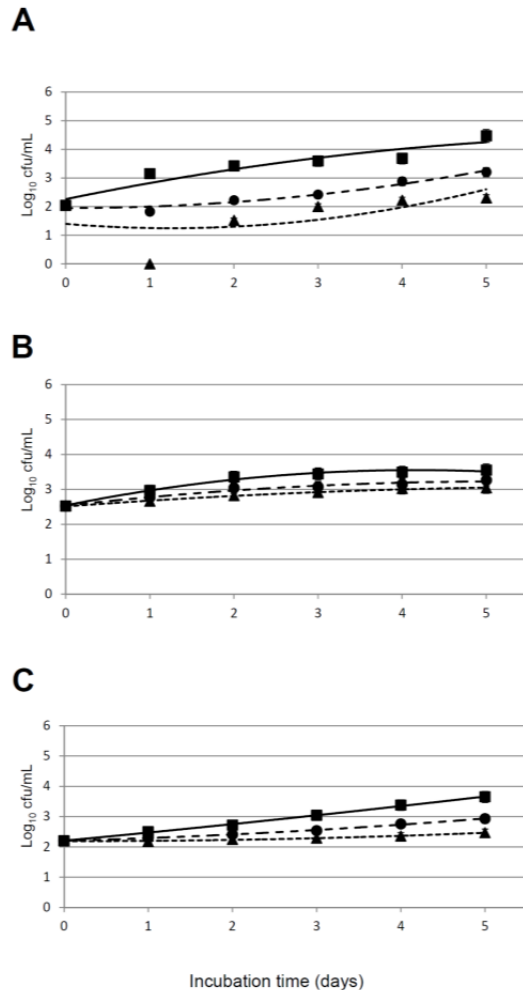


Fig. 2 Growth patterns of *L. monocytogenes* in raw cosmetic milk (A), tanker milk (B) and farm milk (C). For each graph, results are for incubations performed at 15°C, 8°C and 5°C are represented by squares (■), circles (●) and triangles (▲), respectively. Solid and dashed lines indicate trendlines. All data points indicate the average of triplicate viable counts and error bars indicate standard deviations.

Our results for *E. coli* O157:H7 contrast somewhat to those reported by Alhelfi et al. [11] in raw milk samples where pathogen growth and metabolic activity declined progressively at low temperature (4°C), although activity at 20°C increased steadily for 2 days before rapidly declining. However, the growth patterns we observed were not identical between different milk samples, suggesting that the behaviour of pathogens in raw milk is unpredictable and may be dependent on factors (microbiological and otherwise) that are not consistent. Indeed, the initial quality of the milks was highly variable as indicated by the APC results and the presence/absence of indicator microorganisms.

As expected, all spiked bacteria showed the highest rates of growth at 15°C in all milk samples, followed by 8°C, then 5°C. It is important to note that most commercial display and household refrigerators are usually maintained between 6-8°C [10]. Indeed, our own measurements of the temperature of the cosmetic milk bottles at the point-of-sale (using an infra-red temperature probe) indicated that the milk may not have been stored at the recommended conditions with temperatures ranging from 5.4-8.1°C. Similarly, testing of dairy refrigerator cabinets at local retail outlets indicated that the temperatures at the edges of open-style cabinets reached up to 11.6°C. Given the results of our study, storage of raw milk at these conditions would likely compromise the safety of the product.

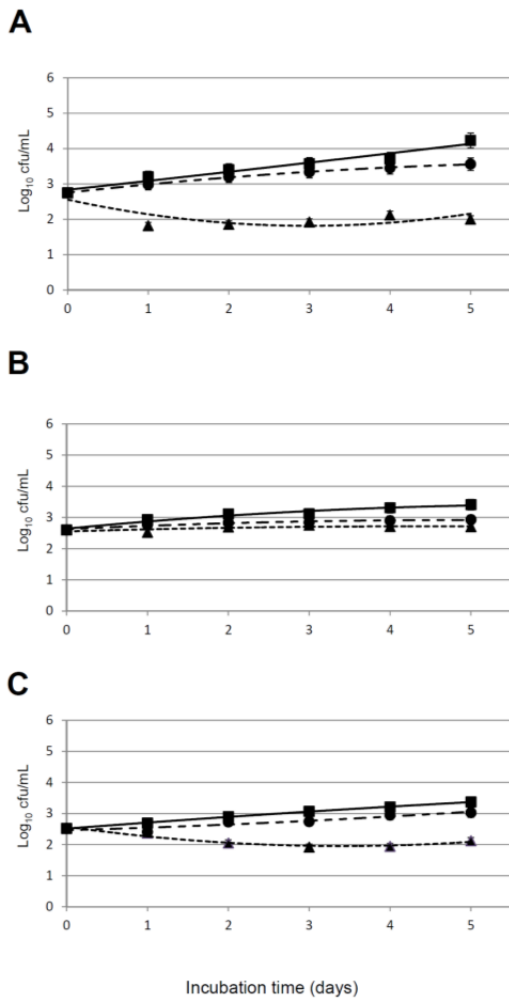


Fig. 3 Growth patterns of *S. enterica* in raw cosmetic milk (A), tanker milk (B) and farm milk (C). For each graph, results are for incubations performed at 15°C, 8°C and 5°C are represented by squares (■), circles (●) and triangles (▲), respectively. Solid and dashed lines indicate trendlines. All data points indicate the average of triplicate viable counts and error bars indicate standard deviations.

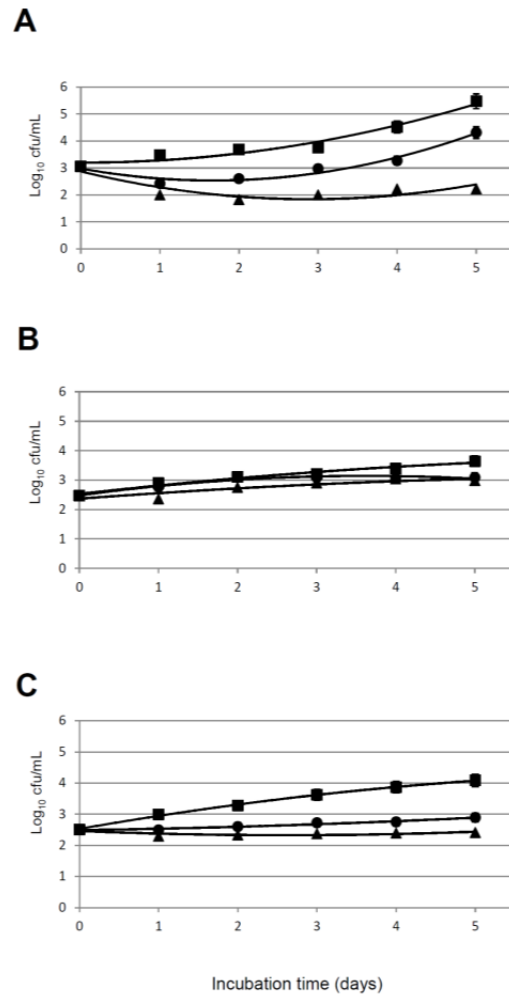


Fig. 4 Growth patterns of *S. aureus* in raw cosmetic milk (A), tanker milk (B) and farm milk (C). For each graph, results are for incubations performed at 15°C, 8°C and 5°C are represented by squares (■), circles (●) and triangles (▲), respectively. Solid and dashed lines indicate trendlines. All data points indicate the average of triplicate viable counts and error bars indicate standard deviations.

4. Discussion

Overall, our results generally indicated that the bacterial populations increased or were at least maintained at the inoculation levels in all spiked milk samples at all temperatures. In some situations (e.g. *Salmonella* and *S. aureus* in cosmetic milk at 5°C), the viable numbers were reduced by approximately 0.5 log_{10} cfu/mL by day 5. However, in no samples were the inoculated bacteria eliminated. These findings validate the concerns that raw milk consumption poses a health risk to consumers and contradict the claims of the raw milk proponents that the “good bacteria” have bacteriostatic and antimicrobial properties and hence are able to prevent the “bad bacteria” from growing in the milk [1]. Given that even low levels of some pathogens can be a safety risk for certain individuals (e.g. immunocompromised people), the risk posed by raw milk consumption is potentially great.

While the results of the present study do not support the claims of raw milk advocates, further research is needed. The milk collections in this study were conducted only during winter and it is documented that season has a significant effect on bacterial contamination of raw milk [12-14]. Therefore, in order to validate the current findings, additional testing should be carried out during different times of the year when the levels of competing raw milk microbiota may be greater or the composition may be different. Indeed, it has been suggested that the autochthonous microbiota of raw milk and raw milk products, when present at high levels and depending on the predominant groups, interfere with the development of foodborne pathogens [15,16]. Bacteria in addition to the strains tested in the current study (such as *Bacillus cereus* and *Campylobacter jejuni*) should also be tested to study their growth and survival in raw milk and their

ability to compete against the existent microbiota. In particular, endospore-forming bacteria may display greater survival characteristics and remain viable in the presence of inhibitory substances produced by milk microbiota.

In summary, this study has not provided evidence to support the claims that raw milk consumption is a low-risk activity. Indeed, the results clearly indicated that the bacterial pathogens were able to survive and proliferate in the presence of resident raw milk microbiota. The importance of maintaining correct temperature during storage of raw milk supports the recommendations of regulators for all dairy products. Given that the cold-chain cannot be guaranteed during raw milk production or storage by consumers and that it is difficult, if not impossible, to eliminate contamination by pathogens, the consumption of such products is clearly associated with risks to human health.

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References

- [1] LeJeune JT, Rajala-Schultz PJ. Unpasteurized milk: a continued public health threat. *Clinical Infectious Diseases*. 2009;48: 93-100.
- [2] U.S. Food and Drug Administration, 2011. Grade “A” pasteurized milk ordinance (2011 Revision). Available at: <http://www.fda.gov>
- [3] Jay-Russell MT. Raw (unpasteurized) milk: are health-conscious consumers making an unhealthy choice? *Clinical Infectious Diseases*. 2010; 51:1418-1419.
- [4] Food Standards Australia New Zealand, 2009a. Raw milk consumer behaviour and attitudes. Available at: www.foodstandards.gov.au
- [5] Knöchel S *Listeria monocytogenes* – not only bad but also persistent. *Australian Journal of Dairy Technology*. 2010;65:50-57.
- [6] Costa Sobrinho P de S, Marçal de Faria CA, Silva Pinheiro J, Gonçalves de Almeida H, Vieira Pires C, Silva Santos A. Bacteriological quality of raw milk used for production of a Brazilian farmstead raw milk cheese. *Foodborne Pathogens and Disease*. 2012;9:138-144.
- [7] Jackson EE, Erten ES, Maddi N, Graham TE, Larkin JW, Blodgett RJ, Schlessner JE, Reddy RM. Detection and enumeration of four foodborne pathogens in raw commingled silo milk in the United States. *Journal of Food Protection*. 2012;75:1382-1393.
- [8] Food Standards Australia New Zealand, 2009b, Microbiological risk assessment of raw cow milk. Available at: www.foodstandards.gov.au
- [9] Oliver SP, Boor KJ, Murphy SC, Murinda SE. Food safety hazards associated with consumption of raw milk. *Foodborne Pathogens and Disease*. 2009;6:793-806.
- [10] Laguerre O, Derens E, Palagos B. Study of domestic refrigerator temperature and analysis of factors affecting temperature: a French survey. *International Journal of Refrigeration*. 2002;25: 653-659.
- [11] Alhelfi NA, Lahmer RA, Jones DL, Williams AP. Survival and metabolic activity of *lux*-marked *Escherichia coli* O157:H7 in different types of milk. *Journal of Dairy Research*. 2012;79:257-261.
- [12] Srairi MT, Benhouda H, Kuper M, Le Gal PY. Effect of cattle management practices on raw milk quality on farms operating in a two-stage dairy chain. *Tropical Animal Health Production*. 2008;41:259-272.
- [13] Grimaud P, Serunjogi M, Wesuta M, Grillet N, Kato M, Faye B. Effects of season and agro-ecological zone on the microbial quality of raw milk along the various levels of the value chain in Uganda. *Tropical Animal Health and Production*. 2009;41: 883-890.
- [14] Mhone TA, Matope G, Saidi PT. Aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. *International Journal of Food Microbiology*. 2011;151: 223-228.
- [15] Dodd CER, Richards PJ, Aldsworth TG. Suicide through stress: a bacterial response to sub-lethal injury in the food environment. *International Journal of Food Microbiology*. 2007;120:46-50.
- [16] Nero LA, de Mattos MR, Barros M de A, Ortolani MB, Beloti V, Franco BD. *Listeria monocytogenes* and *Salmonella* spp. in raw milk produced in Brazil: occurrence and interference of indigenous microbiota in their isolation and development. *Zoonoses and Public Health*. 2008;55:299-305.