EVALUATION OF MICROBIAL QUALITY OF PASTEURIZED MILK SOLD IN HISAR CITY, HARYANA, INDIA

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ABSTRACT

The objective of this study was to evaluate the microbial quality of pasteurized milk in Hisar city in view of regulations under Food Safety and Standards Authority of India act. Sixty pasteurized milk samples from six different brands (A, B, C.D, E and F) were evaluated by estimation of total viable count (TVC), coliform count (CC), Staphylococcus aureus count test (STA) and demonstration of presence or absence of pathogenic microorganism *viz*. *Salmonella* and *Listeria* spp. The result of the study revealed that 35 (58.33%), 36 (60%) and 6 (10%) of 60 samples were having TVC, CC and STA count more than the acceptable limits of Food Safety Standard Authority of India (FSSAI). However, *Salmonella* and *Listeria* spp, were not detected in any of the samples. Majority of the samples screened did not meet the FSSAI recommendations and were of poor quality.

Keywords: Coliform count, FSSAI, *Listeria monocytogenes*, Pasteurized milk, *Salmonella*, Standard plate count

Milk is known to be the complete diet found in nature as it contains fat, protein, carbohydrates, minerals, vitamins and other various ingredients dispersed in water. Although, milk is virtually sterile when secreted into the alveoli of the udder, it gets contaminated during later stages of milk production. As milk is rich in nutrients, it acts as a suitable medium for growth and survival of spoilage as well as pathogenic microbes. Thus, not only shelf life of milk gets reduced, it can also act as an effective vehicle of transmission of disease causing organisms to human beings. In order to increase the shelf life and make it safe for consumers, milk is heat treated in the form of pasteurization before its distribution to the consumer, especially in urban areas of India. However, this pasteruized milk can be safe only if it is properly pasteurized and properly handled and stored during its subsequent distribution. To ensure the quality of pasteurized milk, Food Safety and Standards Authority of India has laid down regulations (FSSAI, 2016) which mention the mandaory requirement of fulfillment of process hygiene and food safety criteria for various milk products including pasteurized milk. Several researchers reported microbiological quality of milk with respect to total viable count (TVC), coliform count (CC), *Staphylococcus aureus* count test (STA) and presence of pathogens (Raj *et al.*, 2010; Surve *et al.*, 2011; Agarwal *et al.*, 2012; Aglawe and Wadatkar, 2012). However, these reports did not mention the status of samples in view of comprehensive requirements of FSSAI regulations. Therefore, the present study was carried out to monitor the microbiological quality of pasteurized milk marketed in Hisar city by taking into consideration FSSAI regulations i.e process hygine criteria (TVC, CC and STA) and food safety criteria (Presence or absence of *Salmonella* spp. and *Listeria monocytogenes*).

MATERIALS AND METHODS

A total of 60 pasteurized milk samples (in the form of 500 mL packets) belonging to six different brands viz A, B, C, D, E and F (10 each) were collected from local market with all aseptic precautions and transported on ice to the laboratory. Samples with valid 'Best before' date were collected only if temperature recorded with the help of infra red thermometer found to be 3-4 °C. Sampling was done during the period starting from August 2018 to February 2019.

University of Vermont enrichment medium (UVM), Fraser enrichment broth, Oxford agar, buffered peptone water, Rappaport Vassilaidis medium, brilliant green agar medium, Oxford Listeria supplement, Simmon s citrate agar, Kovac s reagent, methyl red (MR) reagent, oxidase disc, Gram's staining kit and triple sugar iron agar were procured from the Hi-Media Laboratories Pvt. Ltd., Mumbai.

Tempo® cards for automated enumeration of total viable count (Tempo-AC), total coliform (Tempo-TC), *Staphylococcus aureus* count (Tempo STA) as well as automated biochemical identification of Salmonella and Listeria VITEK® 2 GN & GP ID cards and specific Tempo culture media vials were procured from BioMérieux India Pvt. Ltd.

Total viable count, total coliform count and *Staphylococcus aureus* count was determined by TEMPO® automated test procedure using test specific cards as per manufacturer's instructions.

Emperical standard methods were used to determine presence or absence of pathogens in milk samples viz. Indian Standards Institute (1999) method for Salmonella organisms and Indian Standards Institute (2001) method

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for Listeria organisms was used for preliminary isolation of these pathogens. Suspected isolates were subjected to biochemical confirmation using automated method of VITEK®2 compact system.

RESULTS AND DISCUSSION

Different tests as recommended by FSSAI to analyse the microbiological quality of milk and milk products are total viable count, coliform count, STA count and yeast and mould count for evaluation of process hygiene criteria and detection of food borne pathogens viz: *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus* and Clostridium for evaluation of food safety criteria. In this study, a total of 60 pasteurized milk samples available in local market of Hisar were examined to determine the microbial quality by evaluating Total Viable Count (TVC), Coliform Count (CC), STA count, isolation and identification of *Listeria* spp. and *Salmonella* spp.

Total Viable Count : The TVC is an estimate of the total number of viable aerobic bacteria present in raw and pasteurized milk, which have a negative impact on the quality of milk. As per FSSSI Regulations, the TVC of pasteurized milk should not be more than 3×10^4 cfu/mL. In the present study, of all 60 tested samples, 25 (41.66%) samples showed TVC within the prescribed limit. Whereas, remaining 35 (58.33%) samples failed to meet the regulatory limit. Maximum samples (80%) of A and D showed TVC within permissible limits, while only 20%, 30% and 40% milk samples of brands E, F and C, respectively showed TVC within the permissible limit (Table 1). The TVC in pasteurized milk samples of brands A, B, C, D, E and F were in the range of 3.2×10^2 cfu/ml to 3.9×10^4 cfu/ml, 100 cfu/ml to $>4.9\times10^5$ cfu/ml, 1.5×10^3 cfu/ml to $> 4.9\times10^5$ cfu/ml, 9.9×10^2 cfu/ml to $> 4.9\times10^5$ cfu/ml, 9.3×10^3 cfu/ml to $> 4.9\times10^5$ cfu/ml, 100 cfu/ml to $> 4.9\times10^5$ cfu/ml, respectively.

Results of TVC limits higher as well as lower than in this study have been reported in various studies from different parts of the world. These include a viable plate count of 7.1×10^5 cfu/ml (Shojaei and Yadolahi *et al.*, 2008) from Iran, $>3\times10^4$ to 3.8×10^6 cfu/ml (Nazir *et al.*, 2014) from India, 6.5×10^5 cfu/ml (Elmagli *et al.*, 2006) from Sudan, 1.25×10^5 ml to 9.94×10^5 cfu/ml (Gopi *et al.*, 2001) from Chennai (India), 1.0×10^6 cfu/ml to 2.0×10^7 cfu/ml (Acharya *et al.*, 2017) from Kathmandu (Nepal), 2.94×10^4 cfu/ml (Hussaini *et al.*, 2014) in Beed city in India, 1.9×10^2 cfu/ml to 2.8×10^3 cfu/ml (Rahman *et al.*, 2015) from Dinajpur, Bangladesh. The variability of these counts can be linked to the extent of milk hygiene practices followed during all the steps of production and processing of pasteurized milk in these countries.

Coliform count: Detection of coliform group of bacteria is of much importance than other bacteria because these are indicators of fecal contamination and therefore imply possible presence of other gastrointestinal pathogens. These are destroyed during pasteurization treatment and therefore, a positive coliform test of pasteurized milk indicates either inadequate pasteurization or post-pasteurization contamination. As per the FSSAI recommendations, the acceptable level of coliform microorganisms in the pasteurized milk is ≤10 coliforms/ml. In the present study, only 24 (40 %) samples were found to be acceptable as per the criteria for coliform count. Comparison between brands reveled that, more than 80% samples of brands B, E and F failed to meet regulatory requirement. Most of the samples (90%) of brand D were acceptable (Table 1). The CC in pasteurized milk samples of brands A, B, C, D, E and F was in the range of <10 cfu/ml to 9.1×10^3 cfu/ml, <10 cfu/ml to $>4.9\times10^5$, <10 cfu/ml to $> 4.9\times10^5$ cfu/ml, <10 cfu/ml to 20 cfu/ml, <10 cfu/ml to $> 4.9\times10^4$ cfu/ml and <10 cfu/ml to $>4.9\times10^5$ cfu/ml

Table 1

Evaluation of microbial quality of pasteurized milk with respect to process hygiene criteria

Brands	Total viable count test		Coliform count test		*Staphylcoccus aureus* count test	
	No. of acceptable samples (%)	No of unacceptable samples (%)	No. of acceptable samples (%)	No. of unacceptable samples (%)	No. of acceptable samples (%)	No. of unacceptable samples (%)
A (10)	8 (80)	2 (20)	6 (60)	4 (40)	9 (90)	1 (10)
B (10)	-	10 (100)	1 (10)	9 (90)	10 (100)	0 (0)
C (10)	4 (40)	6 (60)	4(40)	6 (60)	9 (90)	1 (10)
D (10)	8 (80)	2 (20)	9 (90)	1 (10)	8 (80)	2 (20)
E (10)	2 (20)	8 (80)	2 (20)	8 (80)	8 (80)	2 (20)
F (10)	3 (30)	7 (70)	2 (20)	8 (80)	10 (100)	0 (0)
Total (N = 60)	25 (41.66)	35 (58.33)	24 (40)	36 (60)	54 (90)	6 (10)

Note: As per FSSAI criteria, sample is acceptable if a) total viable count is ≤ 3×10^4 cfu/mL and b) coliform count is ≤10 cfu/ml. c) As per TempoR Biomerieux Standards, sample is acceptable if count is ≤10 cfu/ml and unacceptable if count is >10 cfu/ml

respectively (Table 1). Similar observations were recorded by other researchers from various parts of India viz. West Bengal (Chatterjee *et al.*, 2006), Haryana (Nazir, 2011) and Punjab (Agarwal *et al.*, 2012). The findings of present study are comparable with milk quality of other developing countries viz. Iran (Shojaei and Yadollahi, 2008), Sudan (Elmagli *et al.*, 2006) and Nepal (Acharya *et al.*, 2017). In some of the other studies, lower coliform counts in pasteurized milk samples have also been reported (Agalawe and Wadatkar, 2012 and Singh *et al.*, 2015).

***Staphylococcus aureus* count:** The presence of *S. aureus* in milk can cause food poisoning due to production of heat-stable enterotoxin that can withstand heat treatments and which is a major risk to public health. *S. aureus* has traditionally been used as a microbiological indicator of extent of sanitation maintained during processing. In this study, out of 60 pasteurized milk samples which were examined, most of the samples (90%) showed compliance with Tempo Biomerieux (ISO 7218:2007(E) guidelines for *S. aureus* count. In all six brands, number of samples complied with regulatory limit in acceptable limit varied from 8 (80%) to 10 (100%). These findings on STA count are similar to other researchers i.e. 2% samples from Sari city, Iran (Vahedi *et al.*, 2013), 5% samples from Kerala, India (Prejit, 2005), 11.6 % samples from Botswana (Aaku *et al.*, 2004) and 12.5 % samples from Nepal (Acharya *et al.*, 2017).

Compliance status of pasteurized milk samples with respect to process hygiene criteria of FSSAI

As detailed in Table 2, violation of process hygiene criteria of pasteurized milk samples was recorded in 68.33% samples. This indicates lack of hygiene and sanitation during processing of milk or during subsequent transportation and storage of all brands included in the study.

Isolation of *Listeria* spp. and *Salmonella* spp. from pasteurized milk

Listeria monocytogenes and *Salmonella* spp. are

Table 2

Compliance status of pasteurized milk samples as per process hygiene criteria of FSSAI regulations

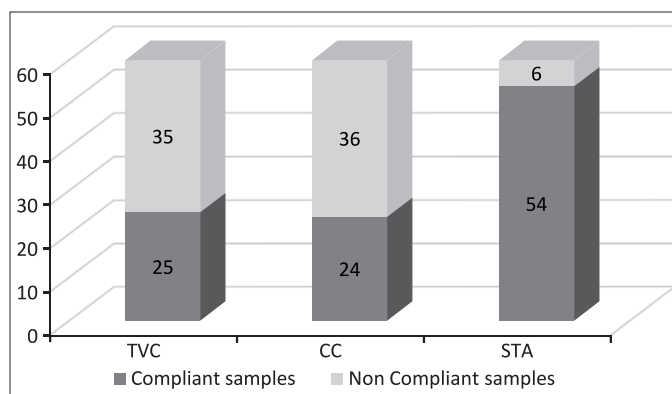| Brands	No. of (%) compliant samples	No. of non-compliant samples (%)
A (10)	5 (50%)	5 (50%)
B (10)	0 (0%)	10 (100%)
C (10)	3 (30%)	7 (70%)
D (10)	7 (70%)	3 (30%)
E (10)	2 (20%)	8 (80%)
F (10)	2 (20%)	8 (80%)
Total (N = 60)	19 (31.66%)	41 (68.33%)

Fig. 1. Compliance of pasteurized milk samples with respect to process hygiene criteria of FSSAI regulations

most important food borne pathogens owing to their ability to cause fatal diseases. Therefore, food safety authorities usually apply stringent regulations for their presence in foods. According to FSSAI standard, Salmonella and Listeria should be absent in 25 gram of the samples. In the present study, out of 60 pasteurized milk samples, none of the sample showed presence of *Listeria* spp. and *Salmonella* spp. Similar to the findings of present study, other researchers also observed the absence of Listeria organisms in pasteurized milk (Harvey and Gilmour 1992; Bhilegaonkar *et al.*,1997; Baek *et al.*, 2000; Agarwal *et al.*, 2012; Rahman *et al.* 2015). Similar results with respect to absence of Salmonella from pasteurized milk were reported in various other studies also (Tangri and Chatli, 2014; Rahman *et al.*, 2015; Munsi *et al.*, 2015 and Singh *et al.*, 2018).

CONCLUSION

From these findings, it can be concluded that there is much scope in the improvement of process hygiene of pasteurized milk belonging to the brands included in the present study. Also there is need to carry out extensive extension education programme for milk producers and processors to strictly follow clean milk production practices.

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