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FULL PAPER

Evaluation of bacteriological quality of locally produced raw and pasteurised milk in Selangor, Malaysia

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Abstract

High demand for milk has been observed amongst the Malaysian public. Hence, research in milk is essential to assure food safety in milk consumption. This study evaluated the quality of locally-produced milk and present of bacterial hazards in cow and goat milk. A total of 120 milk samples including thirty raw cow milk, thirty pasteurized cow milk, thirty raw goat milk and thirty pasteurised goat milk were collected from dairy farms, delivery milkman, marts and markets in Selangor, Malaysia. The bacteriological quality of milk was evaluated for the presence of *Escherichia coli,* mesophilic, and coliform bacteria. An acceptable standard limit of < 1 x 10^5 CFU/mL for the total bacterial count was used to indicate good quality of milk. Overall, all type of milk exceeded 100,000 CFU/mL. The pasteurized raw goat milk showed the highest (7.16 $\log_{10}$ CFU/mL) in total plate count while the pasteurized cow milk recorded as the lowest (5.38 $\log_{10}$ CFU/mL) in total plate count. Approximately half of the milk samples were contaminated with coliform bacteria and a proportion has exceeded the acceptable limit of 50 CFU/mL. The presence of *E. coli* was detected in over 44% of the samples. Milk contaminated with the pathogenic *E. coli* can cause self-limited, watery to bloody diarrhea including severe diseases like haemolytic uremic syndrome (HUS). Hence, it is important to ensure the quality of milk for public health safety.

1. Introduction

In any dairy industries whether small, medium or large scale, milk quality control is essential. Milk consists of high nutritional components that allow rapid multiplication of bacteria especially under unsanitary production and mishandling of milk during storage. The bacteriological quality control is essential to identify the degree of contamination, enumeration of selected microorganisms and ensure that milk complies with the regulatory standards (Chatterjee *et al*., 2006; Muehlhoff *et al.,* 2013). Principally, the scheduled bacteriological assessment of milk and milk products is important for public health protection.

In developing countries, the production of milk is said to be taken place below standard sanitary practices, ineffective farm management and hot tropic weather. All these conditions have contributed to spoilage and economic loss to the milk industry (Worku *et al*., 2012; Yuen *et al*., 2012). Importantly, the nature of milk enables it to be an excellent substrate for the growth of the microorganism. Hence, milk with high quality and safety is not easily accomplished (Worku *et al*., 2012).

While pasteurisation has improved the safety of milk, improper handling can lead to recontamination. The microbial spoilage in pasteurised milk mostly attributed by Gram-negative bacteria. This usually comes from inadequately cleaned and sanitized filling machines (Angelidis *et al*., 2016). Protection of milk after heating can be done through the application of standard hygiene

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in milk processing, right temperature and thermal conditions, and buying high quality of raw milk (Piotrowska *et al*., 2015).

The total plate count (TPC) at 10^5 CFU/mL has been used as the standard by Malaysian regulatory. In addition, coliform and *E. coli* count was also included by Malaysian regulatory for evaluation of microbiological safety in milk. For farmers, high microbial load in milk resulted in higher milk selling price and hence pose economic loss to the local farmers.

The quality of pasteurised milk highly depends on the quality of the raw milk which acts as the starting materials (Angelidis *et al*., 2016). In Malaysia, pasteurised milk has not received sufficient attention and there is no reported data on the bacteriological quality of locally produced pasteurised milk.

Temperature is an important factor for the prevalence and proliferation of microorganism in milk (Reta and Addis, 2015). When milk is subjected to temperature abuse, the microorganisms can multiply to a higher level and may produce toxins. More so, a study from Brazil showed that deficient cold storage chain has contributed to the reduced quality of milk (Petrus *et al*., 2010). Therefore, it is interesting to evaluate the bacteriological quality of milk based on storage temperature at different collection points.

Hence, the aim of this study is to determine the bacteriological quality of cow and goat milk collected in Selangor, Malaysia. The total plate count, coliform count and *E. coli* count of milk samples were evaluated. Both raw and pasteurised milk of the dairy animals was assessed in this study. This study also evaluated the bacteriological quality of milk based on collection points, from farms to marketplaces. The milk samples from marketplaces including milk from mart and market (night market, agro fair and Ramadhan bazaar).

2. Materials and methods

2.1 Sample collection

A total of 120 milk samples comprising thirty raw cow milk, thirty pasteurised cow milk, thirty raw goat milk and thirty pasteurized goat milk. All milk samples were collected from different collection points in Selangor area. This enables milk from different dairy chain being evaluated. The raw milk samples were collected from farms and milkman. The pasteurized milk samples were obtained from the marts and markets which includes night market, Ramadhan bazaar and agriculture fair. The pasteurisation was performed at 60°C for 30 mins. Most of the samples were collected in the morning. Hence, all the milk samples able to be analysed once samples arrived at the lab on the same day.

Approximately 250-500 mL of the milk was aseptically collected and stored in clean bottles or plastic bag. During collection, milk samples were kept in the icebox to maintain chilled conditions and immediately sent to Food Safety and Quality Laboratory 2 in University Putra Malaysia for further analysis.

2.2 Bacteriological analysis

All the milk samples were analysed for the bacteriological quality as according to Fifteen schedule; Regulation 39 in Malaysia Food Act 1983 and Food Regulation 1985. The TPC, coliform count and *E. coli* count were carried out as described by Bacteriological Analysis Manual (BAM) USFDA (BAM, 2001).

For the TPC procedure, Plate Count Agar (PCA) (Sigma-Aldrich, USA) was used. The coliform count was determined using Violet Red Bile (VRB) agar (Sigma-Aldrich, USA). The *E. coli* count was determined using Eosin Methylene Blue (EMB) agar (Merck, Germany).

Initially, 10 mL of the milk sample was dispensed into a sterile stomacher bag containing 90 mL of sterile peptone water. The mixture was homogenised with stomacher for 60 s. The subsequent dilution was prepared in peptone water up to 10^{-6}. An amount of 100 mL of the milk mixture was incubated on the agar plates for 24 hrs at 37°C. The total bacterial count was calculated using colony counter (Galaxy 230).

2.3 Statistical analysis

The means of milk colony counts were analysed using one-way analysis of variance (ANOVA). Statistical significance difference between milk samples type of origin (cattle and goat) and type of milk (raw and pasteurised) were analysed. Minitab 17.0 statistical software (Minitab Inc. Pennsylvania, USA) was used to determine the difference in means of colony count. The data was analysed the data at 95% of confidence interval and 5% level of significance. All the plate counting assays were performed in triplicate of all types of milk samples. The colony counts were presented in $\log_{10}$ CFU/mL.

3. Results

3.1 Milking practices

All of the raw goat milk (n=30) was collected using hand milking, while raw cow milk (n=30) was collected using the line or portable milking. All farmers claimed that they cleaned the udder of the dairy animals prior to milking.

3.2 Total bacterial counts based on milk origins

In this study, all types of milk were contaminated with bacteria and have exceeded the limit set by the Malaysia Food Act 1983 and Food Regulation 1985 (5 $\log_{10}$ CFU/mL). Over 50% of the raw cow milk exceeded the standard, while pasteurised cow milk exceeded at 20%. For raw goat milk, only 23% exceeded the standard and pasteurised goat milk at 47%. From Table 1, the pasteurised goat milk account for the highest mean counts of TPC at 7.16 $\log_{10}$ CFU/mL. The lowest mean TPC was from pasteurised cow milk at 5.38 $\log_{10}$ CFU/mL. The results showed that there are significant differences (p<0.05) in the bacterial loads between different types of milk.

Table 1. Total plate count of bacteria based on milk origins

Type	Origin	Total plate count ($\log_{10}$ CFU/ mL)
Raw	Cow	5.88 ± 1.10^{ab}
	Goat	5.67 ± 1.65^{ab}
Pasteurised	Cow	5.38 ± 1.01^{b}
	Goat	7.16 ± 1.75^{a}

Values are mean±SD. Means with different alphabet superscript are significantly different at p<0.05.

Table 2 shows the mean counts for coliform and *E. coli* from local milk. Nearly 50% of all milk samples were contaminated with coliform. For coliform count, pasteurised goat milk recorded the highest mean counts of contamination at 6.54 $\log_{10}$ CFU/mL. For *E. coli* count, raw cow milk contaminated with 4.70 $\log_{10}$ CFU/mL while pasteurised goat milk was contaminated at the highest mean counts at 6.62 $\log_{10}$ CFU/mL. Pasteurised cow milk recorded the lowest mean count for both coliform and *E. coli* counts. The mean count for coliform and *E. coli* were significantly correlated at r =0.967.

Table 2. Counts of coliform and *E. coli* in milk

Type	Origin	Bacterial count ($\log_{10}$ CFU/mL)	
		Coliform	*E. coli*
Raw	Cow	5.55 ± 1.76^{a}	4.70 ± 1.44^{a}
	Goat	4.59 ± 1.13^{a}	3.92 ± 0.91^{a}
Pasteurised	Cow	3.68 ± 1.32^{a}	3.00 ± 1.14^{a}
	Goat	6.54 ± 1.83^{a}	6.62 ± 1.97^{a}

Values are mean±SD. Means with the same alphabet superscript are not significantly different at p<0.05.

3.3 Total bacterial counts based on collection points

Table 3. Total plate count of bacteria based on the collection points

Type	Origin	Total plate count ($\log_{10}$ CFU/mL)
Raw	Farms	4.62 ± 1.20^{a}
	Milkman	6.17 ± 1.31^{a}
Pasteurised	Market	7.11 ± 1.77^{a}
	Mart	5.35 ± 0.91^{a}

Values are mean±SD. Means with the same alphabet superscript are not significantly different at p<0.05.

From Table 3, the TPC of milk from the delivery milkman, marts and markets have exceeded the limit set by the Malaysia Food Act 1983 and Food Regulation 1985. The milk from marts was at 5.35 $\log_{10}$ CFU/mL. The milk from markets was heavily contaminated with TPC at 7.11 $\log_{10}$ CFU/mL while milk from delivery milkman recorded at 6.17 $\log_{10}$ CFU/mL. On the other hand, milk from farms recorded the lowest at 4.62 $\log_{10}$ CFU/mL.

From statistical analysis, mean counts of raw milk from farms showed significant differences (p<0.05) than raw milk from delivery milkman. However, there is no significant difference (p>0.05) in the mean counts of raw milk with pasteurised milk.

4. Discussion

This study showed that all types of milk were heavily contaminated with bacteria. The TPC is an indicator for monitoring good sanitary practices during milk production, transportation and storage (Worku *et al*., 2012). In general, raw milk produced from healthy dairy animals under hygienic condition should not contain more than 10^4-10^5 CFU/mL of bacteria (O' Connor, 1994). The high microbial load in milk might be linked to poor milking handling, poor animal health services and usage of contaminated water (Giacometti *et al*., 2012; Banik *et al*., 2014).

In this study, the TPC of raw cow milk was 5.88 log $\log_{10}$ CFU/mL. This was lower than the value reported by Chye *et al*. (2004) at 8.2 $\log_{10}$ CFU/mL from local raw cow milk study. In contrast, for local raw goat milk, Suguna and research group reported that a slight lower TPC at 4.5 $\log_{10}$ CFU/mL comparing to current study at 5.67 $\log_{10}$ CFU/mL (Suguna *et al*., 2012).

Contamination can easily occur during the milking process. Insufficient udder cleaning may cause high bacterial contamination (Reta and Addis, 2015). A study in Ethiopia showed that 92% of the farmers have not washed the udder prior to milking and the mean TPC reached up to 1 x 10^{12} CFU/ mL (Abate *et al*., 2016). Besides, it is essential to dry the udder after washing as bacteria can grow in places with moist environment. In according to proper GMP, the correct way is to wash the udder with good quality of lukewarm water and un-fragrance soap and this is yet to be implemented (Gemechu *et al*., 2014).

From observation, some dairy farms from this study were unable to practice good hygiene practices. The farmers did not clean the floor at milking area. This condition allows bacterial contamination from the milking area. Food Hygiene Regulations (2006)

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emphasise that milking area should be clean from any contamination sources like dust, flies, birds and other animals (Gemechu *et al*., 2014). One of the farms from this study allowed their dog to walk by the milking area and thus signify a point of concern. Additionally, farmed animals can be regarded as reservoirs of pathogens. This enables them to potentially transfer pathogens to milk which includes pathogenic *E. coli*, *Staphylococcus aureus*, *Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes* (Farrokh *et al*., 2013).

Temperature has influences high bacterial counts. Milk samples from the market were exposed to a higher temperature in comparing with milk sample from mart as there is no proper refrigerator while selling the milk at the market. Furthermore, Malaysia weather is characterised as warm temperatures (mean >17°C) and abundant rainfall (250-2000 mm). The temperature fluctuates between 23-34°C and relative humidity between 60-95% (Sithambaram and Nizam, 2013). Without right cold chain from farm to processing plant and finally retail points, the bacterial counts can significantly increase together with the warm Malaysia weather.

The pasteurised goat milk in this study was heavily contaminated. Due to heat treatment, this is not expected in pasteurised milk. This can be influenced by poor initial milk quality, defective in pasteurisation machinery, possible post pasteurisation contamination. Post contamination can arise from poor milk processing, unhygienic handling conditions and temperature abuse during storage (Omore *et al*., 2005; Banik *et al*., 2014; Piotrowska *et al*., 2015). Finally, all these factors can contribute to high total bacterial counts.

Some of the pasteurised goat milk was purchased from market. When the mean counts of TPC were analysed based on collection points, it was clear that milk from delivery milkman and market recorded the highest contamination of bacteria. The night market seller from this study used ice to maintain the temperature, which as time passes the ice melted. Besides, milkman delivery from this study selling the milk without putting the milk in the ice container. This highlighted the importance of cold chain at milk sale points.

The pasteurised cow milk, however, showed contamination at only 20% of the milk samples exceed the standard limit. This proves that the pasteurisation is a necessary step and the milk samples lack secondary contamination. This can also be attributed from the use of chilling facilities as some of the pasteurised cow milk was bought from the mart.

According to the Malaysia Food Act 1983 and Food Regulation 1985, the coliform should be less than 50 CFU/mL in raw milk. However, this study showed present of coliform bacteria found in both raw and pasteurised milk. Coliform bacteria is the indicator for faecal contamination in milk. It may also indicate usage of contaminated water, unsanitary milking practices, not maintaining milking equipment and not washed them properly (Banik *et al*., 2014). It is suspected that high coliform count may arise from unsanitary practices during and after milking, rather than faecal contamination. From observation, the dairy goats in this study were reared on raised slatted floored housing system hence can be easily cleaned. The goat dungs can easily fall down through the floor. Additionally, goat's faeces are in pelleted form and easily dried. Therefore, direct faecal contamination is reduced compared to what would occur in cow milk.

Besides that, locally produced - raw cow and goat milk were packed in the plastic container (usually in 250 mL for goat milk and 500 mL for cow milk) from different farms and are available for sale to consumers at small shops or night market (Noted that, after December 2016, no raw milk is legally sold in Malaysia). The usage of plastic container has been associated with high coliform counts in raw milk as plastic is difficult to be cleaned and sterile (Omore *et al*., 2005). Unlike the established dairy companies, they use milk cartoon that suitable are for dairy product packaging. This is yet to be reinforced to the smallholder dairy industries in Malaysia.

In general, the bacterial counts increased as the milk reached the last selling point, market. They sell at high bacterial counts compared to milk from the farm (initial places) and mart (good cold chain system). This reflected from prolonged storage at high temperature. Additionally, Worku and team (2012) agreed that the selling of milk through milkman delivery was commonly practiced with no quality control like no registration system and no communication between authorities and farmers. Hence, the quality of the milk is always subjected to deterioration and imposed a high risk to the consumers.

5. Conclusion

In this study, the finding show bacteriological quality of raw and pasteurised milk of cow and goat milk were not at a satisfactory level. This can be indicated by high bacterial counts from TPC as well as coliform and *E. coli* counts. Pasteurised milk should have lower bacterial counts, but not in this study. Proper refrigeration temperature during transportation, storage

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as well as in farms should be maintained to ensure that milk is at good quality. Among the factors that contribute to low milk, quality are unhygienic handling during milk processing and the absence of cold chains. Training in milk hygiene and quality testing as well as maintaining correct chilling system will be able to improve the quality of milk.

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