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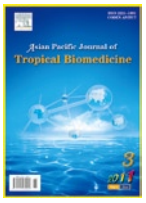

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The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country

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ABSTRACT

Objective: To determine the presence and levels of microbes in unexpired pasteurized milk from randomly selected supermarkets in Kingston, Jamaica. **Methods:** The quantitative study used a stratified random sampling technique in the selection of the 20 representative milk samples from six (6) supermarkets. Microbiological tests such as methylene blue reduction, standard plate count (SPC), coliform plate count (CPC), purity plate culture, gram staining and biochemical tests were performed to examine the microbes in purchased unexpired pasteurized milk. **Results:** One sample (BCr016) had a pH of 4.0, a rancid odour and curdled appearance. It decolourized within one hour during the methylene blue reduction test and was classified as class 4 milk. Seven of the samples were sterile with no microbe growth on the plate count agar and violet red bile salt agar (VRBA). The milk samples that appeared to be safe for consumption were all 10, 11, 12 and 13 days before expiration. The VRBA sample BCr016, had a colony count of 13 400 CFU/ mL. There was the presence of *Escherichia coli* in sample LCr021 which had a standard plate count of 1 580 SPC/mL and a coliform count of 500 CFU/mL. *Enterobacter* sp. was present in colonies from BCr016 and all the other milk samples. **Conclusions:** Unacceptable levels of *Enterobacter* spp. and *Escherichia coli* were found in most of the samples. Effective measures to ensure safe milk for human consumption such as the phosphatase test and methylene blue reduction test should be routinely performed on each batch of milk processed by dairy plants.

1. Introduction

Milk and milk products are excellent high quality foods providing both nutritional and culinary values. However, milk is extremely susceptible to spoilage by microorganisms and the microbiologist plays a major role in the dairy industry in quality control of milk[1]. Cow's milk consists of a variety of nutrients such as fats, proteins, minerals, vitamins, carbohydrates and water and thus it serves as an excellent medium for bacterial growth[2]. Given the appropriate conditions milk can act as a carrier of disease causing microorganisms transformation from cows to humans[1].

Bacteria can be introduced into milk from a wide variety of sources such as workers, infected cows udder, faeces, dust in barns, milk containers or other equipment. Some microbes can serve as disease causing agents when present in milk[3]. Milk can be polluted by *Mycobacterium bovis*, *Brucella species*, *Streptococci* and *Coxiella burnetti* from infected cattle. Agents from human sources such as *Salmonella* species, *Shigella* species, *Corynebacterium diphtheria* and *Streptococcus* species can also be presented in milk. According to Gunasekera[1], psychrotrophic microorganisms are the most important group of microbes present in milk and dairy products. The microbe *Pseudomonas* spp. is considered as the most important psychrotrophs contributing to milk spoilage through production of lipolytic and proteolytic enzymes[4].

According to Prescott *et al*[2], *Campylobacter jejuni* is considered as a leading cause of acute bacterial gastroenteritis in humans. As little as ten of these bacterial cells can lead to the onset of diarrhea and it is transmitted by raw milk[5]. Coliform bacteria include the organisms *Escherichia coli* (*E. coli*) and *Enterobacter aerogenes*, both

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of which are normal inhabitants of the large intestine[3]. The presence of these organisms in milk therefore indicates fecal contamination. The milk can be contaminated by unsanitary handling after the completion of the pasteurization process. *E. coli* is an important food-borne disease organism and enteropathogenic type which can cause diarrhea, even cause complications resulting in fatalities. According to Brock and Madigan[3] approximately 18 000 persons in northern Illinois and surrounding states experienced severe gastrointestinal illness due to infection with *Salmonella typhimurium* in 1985[6]. This outbreak was traced to milk provided by a single dairy plant that was operated by a large grocery store chain. Defective valve in the pasteurizer was detected which lead to improper pasteurization and the presence of Salmonella which caused these serious gastrointestinal complications[6].

Redmond[4] defines pasteurization, as a process of heating a liquid, particularly milk, to a temperature between 55 ℃ and 70 ℃, to destroy harmful bacteria without materially changing the composition, flavor, or nutritive value of the liquid[4]. According to Gunasekera[1] milk pasteurization was introduced as a public health measure in order to destroy human pathogens and to eliminate or reduce the activities of spoilage microorganisms[1]. The viability of bacteria in milk after heat treatments can be assessed by using three different viability indicators: (i) colony forming unit (CFU) on plate count agar, (ii) de novo expression of a *gfp* reporter gene, and (iii) membrane integrity based on propidium iodide exclusion[1]. The methylene blue reduction and phosphatase tests are methods widely used to detect the presence of microbes in pasteurized milk. The standard plate count is used to determine the total number of bacteria present in a specified amount of milk, usually a milliliter (mL). This is used for the grading of milk. The coliform plate count is widely used to determine the total number of coliforms present in one mL of milk sample. This study sought to determine the presence and levels of microbes in unexpired pasteurized milk from selected supermarkets in Kingston, Jamaica.

2. Materials and methods

2.1. Milk collection

Ten supermarkets in Kingston, Jamaica that offers a wide range of grocery and non-grocery items including pasteurized milk to their customers were randomly selected. The unexpired pasteurized milk was stored in small and large refrigerators at 4 ℃ in these supermarkets. The temperatures of these refrigerators were monitored and recorded daily by a member of staff. About 50%-80% of the unexpired pasteurized milk were sold to customers while expired milk were usually returned to their suppliers such as Cremo and Serge Island Diaries.

The selected supermarkets were visited by the investigators in the study. The expiry dates of the milk samples in the large and small refrigerators were noted. A random selection of the milk samples was done covering all observed expiratory dates. The quantitative study used a stratified random sampling technique in the selection of the representative samples. Questionnaires were issued to supervisors at the ten supermarkets in order to collect information on the transportation and storage of the studied milk. Four of the investigated supermarkets did not have any milk in stock when the investigators visited their establishment, therefore, two brands of milk, namely Cremo and Serge Island Dairies, and milk from two different dairy farmers were purchased from the rest six supermarkets that had pasteurized milk in stock. Four Cremo milk cartons with expiration dates of January 17, 20, 25 and 26 and four Island Dairies milk cartons with expiration dates of January 17, 20, 24 and 26 were randomly selected from supermarket A. Four Cremo milk cartons with expiration dates of January 17, 18, 21 and 26 were randomly selected from supermarket B. One Cremo milk carton with expiration date of January 17 and one Island Dairies milk carton with expiration date of January 20 were randomly selected from supermarket C. Two dairy farmers' milk cartons with expiration dates of January 20 and 25 were selected from supermarket D. A Cremo milk carton and one transparent bottled milk with expiration dates of January 16 and 26 were randomly selected from supermarket E. One Cremo milk carton with expiration date of January 20 and one Island Dairies milk carton with expiration date of January 26 were randomly selected from supermarket F. The total number of randomly selected pasteurized milk samples was 20. Samples were collected on the morning of January 14, 2009 and transported on ice to the laboratory, then kept in a refrigerator at 4 ℃ for use in order to maintain similar storage conditions to that of the supermarkets from which they were purchased. The processing of the samples was done in the afternoon of January 14, 2009.

Covert observations were conducted at selected supermarkets. Questionnaires were used to obtain information from six of the investigated supermarkets. Milk samples were cultured and observed for microbial growth. Microbiological tests such as methylene blue reduction, standard plate count, coliform plate count, purity plate culture, gram staining and biochemical tests were used to examine the microbial quality of purchased unexpired pasteurized milk.

2.2. Laboratory determinations

In the methylene blue reduction test, 10 mL of the milk was added to each appropriately labeled tube. One (1) mL of the methylene blue thiocyanate solution was added to the tube; the tube was stoppered and inverted, then tubes were placed in the water bath immediately at 35 ℃. The samples were checked for decolourization after 30 minutes of incubation. Subsequent readings at hourly intervals were then made. After each reading, the decolourized tubes were removed and one complete inversion was slowly made of the remaining tubes. Reduction time was recorded in whole

hours between last inversion and decolourization where decolourization is considered complete when four-fifths of the color has disappeared[5].

In the standard plate count, 9 mL of distilled water was pipetted in each tube and sterilized in an autoclave. Four tubes were labeled undiluted as 10^{-1}, 10^{-2} and 10^{-3} respectively for each milk sample. Under aseptic conditions 2 mL of milk was added to the undiluted tube and a 1 in 10 dilution was made where 1 mL of milk was placed in the tube labeled 10^{-1}. Serial dilutions were then performed and 1 mL was discarded from the10^{-3} tube. One (1) mL of each sample was then transferred to a properly labeled sterile petridish. Approximately 20 mL of plate count agar was then added and the milk samples were mixed thoroughly and uniformly with the agar. The agar was allowed to be solidified and the petri-dishes were then incubated at 37 ℃ for 48 hours. A negative control was done using plate count agar only. The plates were then placed on a colony counter and the number of bacterial colonies was recorded[6].

In the coliform plate count, the procedure was the same as the standard plate count. But approximately 15 mL of violet red bile salt agar (VRBA) was then added to the labeled sterile petri-disk and the milk samples were mixed thoroughly and uniformly with the agar. The agar was allowed to be solidified and an additional 5 mL of VRBA was poured over the surface of the solidified agar mixture. The agar was then allowed to be solidified and incubated. A negative control was done using VRBA only. The plates were then placed on a colony counter and the number of bacterial colonies was recorded[7].

For the purity plate culture, the organisms from the VRBA plate were sub-cultured on blood and MacConkeys agar. Different colony types were seen on the blood and MacConkeys agar for each milk sample, therefore gram stain and biochemical tests were done on each colony type. The standard protocol for gram staining was done. The slide was viewed under the oil immersion objective (× 100)[8]. Biochemical tests for *Enterobacteriaciae* involve the appropriate labeling of biochemical tubes – kligler, urea, citrate and motility indole and lysine (MIL) were labeled appropriately. A flamed inoculation stab was used to touch a colony from the purity plate and was used to inoculate the biochemical tubes. The biochemical tubes were incubated at 37 ℃ for 24 hours. After incubation, the colonies were counted by standard plate count method and the results were recorded[9].

2.3. Statistical analysis

The data obtained from questionnaires were analyzed using Statistics Package for Social Sciences (SPSS) software and Microsoft Excel 2007. This test combines ANOVA with comparison of differences between means of the treatments at the significance level of $P< 0.05$.

3. Results

Nineteen of the twenty milk samples collected had a pH of 7.0, a normal odour and homogenous appearance. The other sample BCr016 collected on January 14, 2009 had a pH of 4.0, a rancid odour and curdled appearance. Except sample BCr016 decolourized within one hour during the methylene blue reduction test and was classified as class 4 milk; all the other 19 samples were not decolorized and were ranked as class 1 products. (class 1-excellent, not decolorized in 8 hours; class 2-good, decolorizied in 6-8 hours; class 3- fair, decolorized in 2-6 hours; class 4-decolorized in less than 2 hours.)

The standard plate count and coliform plate count were performed where both controls passed; hence the results were accepted as being valid. Further testing through the standard plate count and coliform plate count revealed that seven of the samples were sterile, that is, no growth was found on the plate count agar and violet red bile salt agar. These samples were SCr026, SCr025, SID024, LCr026, BCr026, PIDe026 and SID026 (Table 1, 2). These results correlated well with the methylene blue reduction results which decolourized after 8 hours and were classified as excellent quality for human consumption (class 1). The milk samples that appeared to be safe for consumption were all 10, 11, 12 and 13 days before expiration.

No colonies were formed on the standard plate count agar for sample BCr016. However on the VRBA a coliform plate count of 13 400 CFU/mL was obtained (Table 3). There were other samples that had high coliform plate count. These are: SCre017 with a coliform plater count of 10800 CFU/mL, SCr017 with a count of 1 050 CFU/mL, LCr021 with count of 500 CFU/mL, SID020 and SIDe020 had counts of >300 CFU/mL and the other samples having counts less than or equal to 38 CFU/mL (Table 2).

Colonies from CDa025, LCr017, BCr016, on the VRBA that was subjected to culturing on blood agar and MacConkey's agar, gram staining, and biochemical testing revealed the presence of lactose fermenting, gram negative bacilli which were identified as an Enterobacter species (Table 3). Gram staining revealed that gram negative bacillus was identified as *E. coli* in sample LCr021.

The majority (80%) of the supermarkets indicated that they have had cases of early spoilage of pasteurized milk while the remaining 20% stated that they have never had premature milk spoilage. One-fifth (20%) of the supermarkets usually have spoilage of milk one day before expiration while 40% of the supermarkets had their milk spoiling two days before the expiration date. There was spoilage of milk three days before the expiration date at 20% of the supermarkets.

One-tenth (10%) of the respondents stated that improper processing of milk could account for premature spoilage of milk at their supermarket; 30% stated that contamination could be a contributing factor for early milk spoilage, while 20% of the responses pointed to a change in temperature during transportation and refrigeration respectively as possible causes of early milk spoilage.

The majority (80%) of the respondents returned spoiled milks to the supplier and none of them disposed of the

Table 1
Standard plate count of unexpired pasteurized milk samples.

Identification number	Undiluted	10^{-1}	10^{-2}	10^{-3}	Actual plate count (per mL)
SCr017	56	23	68	14	56
SCr026	0	0	0	0	0
SID020	>300	>300	105	19	10 500
SID026	0	0	0	0	0
CDa025	>300	>300	150	94	94 000
LCr021	>300	158	21	0	1 580
LCr017	220	20	50	1	500
BCr016	0	0	0	0	0
SCre017	>300	42	8	0	420
SIDe020	>300	124	8	0	1 240
SCr020	58	36	11	0	360
SCr025	0	0	0	0	0
SID017	169	78	18	6	780
SID024	0	0	0	0	0
CDa020	33	13	6	0	33
LCr026	0	0	0	0	0
LCr018	>300	223	168	79	79 000
BCr026	0	0	0	0	0
PCre020	19	5	0	0	<30
PIDe026	0	0	0	0	0

Table 2
Coliform plate count of unexpired pasteurized milk samples.

Identification Number	Undiluted	10^{-1}	10^{-2}	10^{-3}	Actual plate count (per mL)
SCr017	>300	105	21	9	1 050
SCr026	0	0	0	0	0
SID020	>300	>300	>300	>300	>300
SID026	0	0	0	0	0
CDa025	>300	20	16	3	>300
LCr021	68	50	26	13	500
LCr017	30	3	0	0	30
BCr016	>300	>300	134	9	13 400
SCre017	>300	>300	108	0	10 800
SIDe020	>300	26	1	0	>300
SCr020	16	5	0	0	<30
SCr025	0	0	0	0	0
SID017	38	12	3	0	38
SID024	0	0	0	0	0
CDa020	13	4	0	0	<30
LCr026	0	0	0	0	0
LCr018	27	12	9	3	<30
BCr026	0	0	0	0	0
PCre020	9	5	0	0	<30
PIDe026	0	0	0	0	0

Table 3
Colonial morphology, gram stain and biochemical results of milk samples from the supermarkets.

Identification number	Isolate	Blood agar	MacConkey's agar	Gram stain	K	U	C	M	I	L	Organism identified
SCr017	A	circular dull a-hemolytic colonies	Irregular flat dull mucoid LF colonies	Small GNB in pairs	A/ A-G	+	-	-	-	-	*Enterobacter* species
	B	Cream non hemolytic colonies	Irregular flat dull mucoid LF colonies	Small GNB in pairs	A/A-G	-	+	-	-	-	*Enterobacter* species
	C	Small grey shiny opaque butyrous a-hemolytic colonies	Small raised shiny LF colonies	Short GNB	A/A	-	-	-	-	-	*Enterobacter* species
SID020	A	Cream non hemolytic colonies	Irregular flat dull mucoid LF colonies	Large thick GNB	A/A	-	+	-	-	-	*Enterobacter* species
	B	Round cream flat circular dull a-hemolytic colonies	Irregular flat dull mucoid LF colonies	Large thick GNB	A/A-G	-	+	-	-	-	*Enterobacter* species
SCre017	A	Small cream colonies non hemolytic	Irregular flat dull LF colonies	GNB	A/A-G	-	+	-	-	-	*Enterobacter* species
	B	Small cream circular raised opaque dull non-hemolytic colonies	Small raised shiny LF colonies	GNB	A/A	-	+	+	-	-	*Enterobacter* species
SCr020	A	Small circular raised,cream a-s hemolytic colonies	Raised mucoid LF colonies	Short GNB	A/A	-	+	+	_	-	*Enterobacter* species
	B	Small circular raised opaque dull B-hemolytic colonies	Irregular flat dull LF colonies	Short GNB	A/A	-	-	+	-	-	*Enterobacter* species
SID017	A	Small circular raised,cream non hemolytic colonies	Large irregular shiny butyrous LF colonies	GNB	A/A	+	+	+	-	-	*Enterobacter* species
	B	Irregular raised cream shiny butyrous a-hemolytic colonies	Large irregular flat dull LF colonies	GNB	A/A	+	+	+	-	-	*Enterobacter* species
CDa025	A	Cream non hemolytic colonies	Raised mucoid LF colonies	Short GNB	A/A	-	+	+	_	+	*Enterobacter* species
	B	Small circular raised opaque dull B-hemolytic colonies	Irregular flat dull LF colonies	Short GNB	A/A-G	-	-	+	-	-	*Enterobacter* species
LCr021	A	Large circular raised beta hemolytic colonies	Raised mucoid LF colonies	GNB	A/A	-	-	+	+	+	*Escherichia coli*
	B	Large cream convex circular opaque dull a-hemolytic colonies	Large irregular flat dull LF colonies	Short GNB	A/A	-	-	-	-	-	*Enterobacter* species
LCr017	A	Cream circular non hemolytic colonies	Convex circular shiny butyrous LF colonies.	GNB	A/A-G	+	+	+	-	+	*Enterobacter* species
	B	Small beta hemolytic colonies	Irregular raised dull serrated LF colonies	Large thick GNB	A/A	+	+	-	-	-	*Enterobacter* species
	C	Small circular raised opaque cream colonies	Irregular raised dull serrated LF colonies	Short thick GNB	A/A	-	-	-	-	-	*Enterobacter* species
BCr016	A	Small circular raised,cream non hemolytic colonies	Large irregular shiny butyrous LF colonies	GNB	A/A	+	+	+	-	-	*Enterobacter* species
	B	Irregular raised cream shiny butyrous a-hemolytic colonies	Large irregular flat dull LF colonies	GNB	A/A	-	+	+	-	-	*Enterobacter* species
CDa025	A	Cream non hemolytic colonies	Raised mucoid LF colonies	Short GNB	A/A	-	+	+	_	+	*Enterobacter* species
	B	Small circular raised opaque dull B-hemolytic colonies	Irregular flat dull LF colonies	Short GNB	A/A-G	-	-	+	-	-	*Enterobacter* species

K: kligler; C: Citrate; I: Indole; U: urea; M: motility; L: Lysine.

spoiled milk. One-tenth (10%) of the supermarket indicated that customers' complaint of purchasing spoiled unexpired milk while 70% of respondents did not receive any complaints from customers of purchasing spoiled unexpired milk.

4. Discussion

The pH informs precisely about the freshness state of milk and as fresh milk is neutral or has slightly acid tendency, the action of lactic bacteria will decrease the pH[10]. Nineteen of the twenty milk samples collected had a neutral pH, normal odour and homogenous appearance. This gave an indication that these milk samples were not spoiled. Sample BCr016 which was collected on January 14, 2009 was spoiled two days before its expiration date of January 16, 2009. Spoilage was evidenced by curdling, a rancid odour and an acidic pH of 4. The acidic pH is due to the production of lactic acid from lactose present in the milk by spoilage microorganisms. When the acidity increases in the milk, groups of casein proteins lose their negative charges and their ability to repel each other. They then bond with each other, causing coagulation, or curdling of the milk[11].

Sample BCr016 decolourized within one hour during the methylene blue reduction test. It was therefore classified as class 4 milk which is defined as poor quality milk based on the acceptable standard which states that milk decolourized in less than two hours is classified as poor (class 4). The short time taken for decolourization of the methylene blue is an indication of the high microbial load present in milk therefore rendering the milk unsafe for consumption. All other milk samples did not decolourize until after eight hours and were thus classified as class 1 milks. These were regarded as excellent for consumption based on acceptable standard which states that milk not decolourized in eight hours is excellent.

The standard plate count and coliform plate count were performed where both controls passed; hence the results were accepted as being valid. Further testing through the standard plate count and coliform plate count revealed that seven of the samples were sterile, that is, no growth was found on the plate count agar and violet red bile salt agar (VRBA). These samples were SCr026, SCr025, SID024, LCr026, BCr026, PIDe026 and SID026. These results correlated well with the methylene blue reduction results which decolourized after 8 hours and were classified as being excellent quality for human consumption. The milk samples that appeared to be safe for consumption had 10, 11, 12 and 13 days before expiration. The pasteurization techniques used for these samples appeared to be adequate.

No colonies were formed on the standard plate count agar for sample BCr016, however on the VRBA a colony count of 13 400 CFU/mL was obtained. This is an extremely high coliform count which is greater than the acceptable count of 10 CFU/mL as defined by Standard Methods for the Examination of Dairy Products (SMEDP) in 1993[12]. This high microbial activity correlates well with the decolourizing time of less than one hour in the methylene blue reduction test. Colonies from BCr016 on the VRBA were subjected to culturing on blood agar and MacConkey's agar, gram staining, and biochemical testing. These tests revealed

the presence of lactose fermenting, gram negative bacilli which were identified as an *Enterobacter* sp. based on the biochemical results. *Enterobacter* species are members of the Enterobacteriaceae family whose presence in milk indicates fecal contamination since they are inhabitants of the large intestine[13].

The results of the microbiological tests conducted revealed the presence of *E. coli* in sample LCr021. This sample had a standard plate count of 1 580 SPC/mL and a coliform count of 500 CFU/mL. The standard plate count limit for pasteurized milk is 20 000 SPC/mL and the coliform plate count limit is 10 CFU/mL[14]. Therefore the standard plate count is acceptable as it is less than 20 000 SPC/mL but the coliform count is unacceptable since it exceeds the acceptable limit of 10 CFU/mL. Colonies of LCR021 from VRBA showed beta haemolytic colonies on blood agar and lactose fermenters on macConkey's agar. Gram staining revealed gram negative bacilli that were identified as *E. coli* based on biochemical results.

Higher levels of index organisms often do not, but may correlate with a greater probability of enteric pathogen(s) pollution and the absence of the index organism does not always mean that enteric pathogens are absent from the food[9]. However, index organisms such as *E. coli* are still being utilized as indicators for the overall quality of food and hygienic conditions present during food processing. The counts obtained are used as an assessment of the adequacy of pasteurization of milk. The cartoon containing milk sample LCR021 from one of the supermarkets was intact and there was no observed sign of being tampered. The presence of *E. coli* in LCr021 may be due to inadequate pasteurization, poor hygienic processing conditions and/or post processing contamination of the milk because proper pasteurization inactivates levels of *E. coli* anticipated in raw milk[15].

E. coli and other Enterobacteriaceae are common in food manufacturing environments and may become part of the resident microflora of the facility especially when sanitation is insufficient. It is also possible for *E. coli* to grow on some foods under refrigeration[7]. This makes it important for the pasteurization process to adequately elimination of any existing *E. coli* and other organisms from milk. The low infectious dose of *E. coli* makes a serious health risk, as a small amount of *E. coli* consumed in milk can cause serious gastrointestinal complications. Even slight contamination of surfaces or work areas may cause serious infection[16]. In this way, there are implications for a wide range of food handling and production industries including abattoirs, dairies, chilled food counters in supermarkets, salads and chilled food preparation factories[17]. With such a significant coliform count and the characteristic low infectious dose of *E. coli*. The presence of *E. coli* in LCr021 could be the culprit of gastrointestinal illnesses if consumed. Strains of *E. coli* capable of causing gastroenteritis include enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohemmorrhagic (EHEC) and enteroaggregative (EAggEC) [18]. No further tests were however employed to identify individual strains, since *E. coli* is a pathogenic coliform capable of producing gastrointestinal illness regardless of the strain. An initial attempt was made to trace the source of the *E. coli* in LCr021 by having dialogue with the staff at the supermarket however we were unable to find the source of the contamination.

Observation of the supermarket from which LCr021 was purchased revealed a small refrigerator which allows customers to conveniently open and close. The milk was stored at a suitable temperature but the atmosphere of the supermarket was very hot which could possibly result in heat entering the refrigerator. This can facilitate the multiplication of existing microorganisms leading to an unacceptable high microbial count in milk.

According to SMEDP (1993)[12] samples CDa025 (94 000 SPC/mL) and LCr018 (79 000 SPC /mL) were found to have an unacceptable high microbial load. On the basis of comparison with SMEDP, samples SCr017 (1 050 CFU/mL), SID020 (>300 CFU/mL), CDa025 (>300 CFU/mL), LCr017 (30 CFU/mL), BCr016 (13 400 CFU/mL), SIDe020 (>300 CFU/mL) and SID017 (38 CFU/mL) were found to contain an unacceptable number of coliforms. These coliforms were further identified as *Enterobacter* species. The presence of *Enterobacter* spp. in the milk samples is not a significant pathological finding. Primary infections caused by *Enterobacter* spp. are rare in immuno-competent patients. Infections are more commonly found in hospital acquired infections of neonates and the immunocompromised[19]. Since *Enterobacter* spp. are not a significant pathological finding no further tests were employed to identify the individual species. Both standard plate count and coliform count of PCre020 were acceptable therefore it was not relevant to proceed with the identification of organisms within this sample. The sample can therefore be said to be safe for consumption and the pasteurization technique is adequate.

Standard plate count of milk that has been freshly pasteurized is generally 500 SPC/mL. This initial standard plate count most often reflects the level of thermoduric bacteria that is, those able to survive the heat treatment during pasteurization[20]. Initial counts greater than 1 000 SPC/mL suggest a potential contamination problem either in the raw milk supply or within the processing equipment[20]. Data obtained from supermarkets revealed that pasteurized milk is transported on refrigerated trucks to maintain the shelf life of the product. All of these supermarkets store milk at 4 ℃ and 80% complained of having cases of premature milk spoilage. According to supermarket personnel, the possible causes of spoilage include malfunctioning of refrigerators, contamination, improper processing and change in temperature during transportation. The majority (70%) of the supermarkets did not receive customers' complaint of early spoilage of milk while 10% received complaints; however none of the supermarkets in the study

received customers complaint of developing gastrointestinal illness after the consumption of pasteurized milk purchased from their business.

The microbial content of unexpired pasteurized milk in this study is unacceptably high as significant amounts of bacteria including coliforms were found in pasteurized milk processed at different dairy plants. Even though significant amount of coliforms were identified, Enterobacter species were most frequent present. Seven of the samples appeared to be sterile but interestingly, *E. coli*, a causative agent of gastrointestinal illnesses was found in one of the samples processed. Premature spoilage of milk is also a common finding however most milk becomes spoiled closer to the expiration date. Spoilage could perhaps be due to changes in temperature, improper pasteurization or post contamination due to unsanitary practices. Defective pasteurization, adulteration of pasteurized milk with raw milk and unsanitary handling are all contributory factors to early milk spoilage. Pathologically significant organisms may enter milk after pasteurization leading to severe infections and gastrointestinal illnesses following human consumption. The key to preventing spoilage and prolonging the shelf life of milk products is to prevent post-pasteurization contamination through well designed quality assurance. It is also the key responsibility of both consumers and suppliers to adequately store milk at suitable temperatures in order to control the levels of microorganisms and to retard the rate of milk spoilage. Effective measures to ensure safe milk for human consumption such as the phosphatase and methylene blue reduction tests should be routinely performed on each batch of milk processed by dairy plants. Medical examination of milk handlers should also be done to reduce milk contamination by infected handlers.

Conflict of interest statement

We declare that we have no conflict of interest.

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