A comparison of selected public health criteria in milk from milk-shops and from a national distributer

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ABSTRACT
Selected public health criteria of pasteurised milk available to the consumer from milk-shops in a pre-defined area of Pretoria compared with a national distributor’s milk was evaluated. Of the 135 milk samples purchased from milk-shops, 87 % were not fit for human consumption on the basis of the minimum standards prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). The national distributor’s milk (n = 79) did not contain any pathogens, toxins nor inhibitory substances and passed all the criteria laid down in the Act. Even though milk-shop milk was sold as having been pasteurised, 38.5% of samples were alkaline phosphatase positive, indicating probable inadequate pasteurisation. Milk-shop milk quality varied between milk-shops and between sampling days and differed significantly (P < 0.05) from the national distributor’s milk. Total aerobic plate and coliform counts were generally high for all milk-shop milk samples. Somatic cell counts of milk-shop milk differed significantly (P < 0.05) from the national distributor’s milk. *Escherichia coli* was detected in 1 ml of 17 % of milk-shop milk, 95 % of which originated from milk which was alkaline phosphatase positive. *Salmonella* spp. could not be detected in 1 ml in any of the *E. coli*-positive milk tested. *Staphylococcus aureus* was isolated from 40 % of milk-shop milk samples, and *S. aureus* enterotoxins from 7.8 % of 51 cultures. Inhibitory substances were detected in 54.1 % of milk-shop milk. The presence of inhibitory substances and the isolation of *E. coli* and *S. aureus* (some of which were able to produce enterotoxins) indicated potentially unsafe milk and poses a serious public health risk to consumers.

Key words: milk hygiene, milk-shops, national distributor, pathogens, Pretoria, toxins, veterinary public health.


INTRODUCTION
Cow milk is a highly nutritious and valuable human food, but its nutrient composition also makes it an ideal medium for bacterial growth, especially in the lower socio-economic sectors and the isolation of *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter* spp. and *Salmonella* spp., especially since mass production and distribution of raw milk-borne diseases are probably at least as dangerous as pathogenicity, toxigenicity and shelf-life, other bacteria are pathogenic to consumers before consumption, and therefore contaminated milk is potentially more dangerous.

There have been numerous outbreaks of milk-borne diseases in humans with pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter* spp. and *Salmonella* spp., especially since mass production and distribution brought about a dramatic change in public awareness of milk safety.

Outbreaks of disease from milk have been reported in South Africa since the late 1980s. A recent study [1], conducted in 1999, found that 21% of milk samples from supermarkets were positive for *Salmonella* spp. and *Escherichia coli*, respectively. These findings are consistent with previous studies conducted in other countries [2,3]. The results of this study highlight the need for continued monitoring of milk quality in South Africa.

The aim of this study was to evaluate the safety and shelf-life of pasteurised milk available to the consumer in a predetermined area of Pretoria, comparing 2 different marketing systems. Firstly, milk purchased from ‘milk-shop’ distributors who buy milk from farmers on volume alone, with no incentives paid for quality, was evaluated. Processing and packaging took place at a plant under strict hygienic conditions before distribution. Secondly, milk purchased from ‘milk-shop’ distributors who buy milk from farmers on volume alone, with no incentives paid for quality, was evaluated. Processing and packaging took place at a plant under strict hygienic conditions before distribution. The study was conducted on raw milk samples collected from milk-shops and from a national distributor.

MATERIALS AND METHODS
Study design
One hundred and thirty-five milk samples were obtained over a 6-week period from June to August 1998 from 4 randomly chosen milk-shops (Milk-shops 1, 2, 4 and 5) and from 1 selected milk-shop (Milk-shop 3). Seventy-nine samples of milk, originating from a well-known national distributor’s commercial brand of milk were purchased from 3 supermarkets (Supermarkets 1, 2 and 3), and were used as the reference control milk. Milk-shop 3 and Supermarket 3 were situated on the same premises, selling both milk originating from a bulk tank as well as milk from the national distributor. This outlet was


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chosen because the management of the milk with respect to the cold chain should have been the same as both types of milk were kept in the same display cabinet. All shops were situated in the northwestern parts of Pretoria.

**Laboratory procedures**

The temperature of the milk was taken within 5 minutes of purchase by decimal- ing approximately 100 ml of milk into a separate plastic container and measuring the temperature using a calibrated electronic thermometer. The decanted milk was then discarded. The balance of the milk was kept on ice in a cool box until it was analysed in the laboratory. All microbiological analyses were carried out within 4 hours of the milk being purchased. Milk samples were kept in a household refrigerator until they were processed.

Standard procedures for the use of 3M Petrifilm aerobic count plates were used for aerobic colony count, and plates were incubated at 32 °C for 48 hours. Standard procedures for the use of the 3M Petrifilm E. coli-coliform count plates were used for the E. coli counts. An incubation temperature of 32 °C, and not 35 °C as prescribed by the Petrifilm manufacturers, was used as this was according to the method described in the Act17. Coliform counts were evaluated using 3M Petrifilm rapid coliform count plates. Single-use disposable pipettes were used for each of the serial dilutions.

The Aschaffenburg and Mullen alkaline phosphatase test was performed, using standard methods as described in the Act17. The somatic cell count was determined using the Fossomatic apparatus, using standard operating procedures. Antibiotics and other antimicrobial residues were tested for using the Brilliant Black Reduction Test (Laboratorium Enterotox, Germany) following standard procedures17. The Brucella milk ring test was used to identify B. abortus antibodies in milk using the standards compiled by the South African Institute of Medical Research.

Staphylococcus aureus isolation was done on Baird Parker Agar Base. A positive colony was confirmed as being S. aureus by means of the Staphylase test (Oxoid Limited, Basingstoke, Hampshire, England). Colony-forming units were not enumerated. Discrete S. aureus colonies were subcultured onto Tryptone Soya Broth and incubated overnight at 37 °C, and subsequently tested for the presence of Staphylococcal enterotoxins A, B, C, and D by means of reversed passive latex agglutination, using the SET-RPLA Staphylococcal enterotoxin test kit (Oxoid). The Staphylococcal enterotoxin test was done on all positive S. aureus cultures. Fifteen milk samples from the national distributor were also tested, 1 from each day of sampling. These samples were centrifuged for 15 minutes at 3400 rpm and the sediment was discarded. Enterotoxin detection was carried out on the supernatant.

**Data analysis**

Data were analysed using the statistical computer package SAS (SAS Institute Inc., NC). Sigma Plot (Jandel Scientific) was used to generate the graphs. Data on bacterial enumerations were converted to log10 values because of their non-normal distribution. Significance was accepted at P < 0.05.

**RESULTS AND DISCUSSION**

The temperature of milk was below 5 °C in only 26.6 % of samples purchased (Table 1). Maintenance of the cold chain is an important factor influencing the safety and keeping quality of milk, especially in a country with a warm climate like South Africa. To delay the growth of microorganisms, it is recommended to hold the milk at ≤ 5 °C.23 Lück et al.24 reported that when the storage temperature is increased to 7 °C the standard plate count of a milk sample after 7 days may be as much as 1000 times higher than on a comparable sample stored at 4–5 °C. Gruetzmacher and Bradley4 cited several authors who found that a 3 °C rise in temperature decreases the shelf-life of milk by half. The normal cold chain can, however, only contribute to a limited improvement of the shelf-life of pasteurised milk when the products contain large numbers of post-processing contaminants which grow at cold chain temperatures5. At elevated temperatures the growth of pathogenic organisms such as S. aureus, Bacillus spp. and enterotoxin-producing E. coli is increased and can therefore cause health hazards3.

Fifty-two of the 135 milk-shop milk samples tested (38.5 %), were alkaline phosphatase positive indicating inadequate pasteurisation (Table 1)23. One of the 2 milk-shops with alkaline phosphatase positive samples, had no negative alkaline phosphatase results over the entire 6-week period. Significantly, all the milk-shops in the study had a High-Temperature-Short-Time (HTST) pasteuriser present and displayed ‘Pasteurised milk’ signs. The national distributor’s milk was always alkaline phosphatase negative. Milk-shops 1 and 4 either did not pasteurise at all or the pasteuriser did not work efficiently. The fault in pasteurisation was an ongoing problem over a 6-week period. The Act states that if pasteurisation is carried out according to the high-temperature short-time method, thermographic recordings of pasteurisation temperatures must be made and kept for at least 4 weeks, and the apparatus used must be calibrated monthly23. A positive alkaline phosphatase result may also indicate the possible addition of raw milk to pasteurised milk or reactivation of the phosphatase enzyme by high bacterial numbers in the milk.

Table 1: Temperature of the milk (°C) at the time of purchase and potential hazards present in the milk.

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of samples tested</th>
<th>Range (°C)</th>
<th>Number ≤ 5 °C (%)</th>
<th>ALP positive</th>
<th>E. coli positive in 1 ml</th>
<th>S. aureus positive in 1 ml</th>
<th>Positive for inhibitory substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk-shop 1</td>
<td>27</td>
<td>3.5–10.5</td>
<td>13 (48.1)</td>
<td>27</td>
<td>21</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>Milk-shop 2</td>
<td>27</td>
<td>4.0–7.5</td>
<td>9 (33.3)</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Milk-shop 3</td>
<td>27</td>
<td>5.0–10.0</td>
<td>1 (3.7)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Milk-shop 4</td>
<td>27</td>
<td>6.5–11.0</td>
<td>0 (0)</td>
<td>25</td>
<td>2 (11)</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Milk-shop 5</td>
<td>27</td>
<td>4.5–9.0</td>
<td>3 (11.1)</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Supermarket 1a</td>
<td>25</td>
<td>1.5–7.0</td>
<td>20 (80.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Supermarket 2a</td>
<td>27</td>
<td>2.5–8.5</td>
<td>9 (33.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Supermarket 3a</td>
<td>27</td>
<td>4.5–8.0</td>
<td>2 (74.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Milk from the same national distributor, purchased at 3 different outlets.

1 ELISA = alkaline phosphatase.

2 E. coli suspect.

23 The temperature of milk was below 5 °C in only 26.6 % of samples purchased (Table 1). Maintenance of the cold chain is an important factor influencing the safety and keeping quality of milk, especially in a country with a warm climate like South Africa. To delay the growth of microorganisms, it is recommended to hold the milk at ≤ 5 °C. Lück et al. reported that when the storage temperature is increased to 7 °C the standard plate count of a milk sample after 7 days may be as much as 1000 times higher than on a comparable sample stored at 4–5 °C. Gruetzmacher and Bradley cited several authors who found that a 3 °C rise in temperature decreases the shelf-life of milk by half. The normal cold chain can, however, only contribute to a limited improvement of the shelf-life of pasteurised milk when the products contain large numbers of post-processing contaminants which grow at cold chain temperatures. At elevated temperatures the growth of pathogenic organisms such as S. aureus, Bacillus spp. and enterotoxin-producing E. coli is increased and can therefore cause health hazards.

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Standard plate counts or total aerobic colony counts are used to estimate viable bacterial populations in the pasteurised
milk and reflect the hygienic practices used in the production, processing and handling of the milk. They give a crude indication of the milk’s shelf-life. Figure 1 shows that the standard aerobic plate count for milk-shop milk ($n = 129$) varied greatly over the 6-week sampling period. Counts ranged from $1.0 \times 10^2$ to $2.66 \times 10^7$ cfu/ml, with a median value of 41 000 cfu/ml (legal limit <50 000 cfu/ml$^2$). Individual samples, however, showed that 74 % of samples had counts lower than 50 000 cfu/ml and were therefore within the legal limits. Standard aerobic plate counts for the national distributor’s milk ($n = 79$) varied from 700 to 8700 cfu/ml with a median count of 2200 (Fig. 1). The standard plate counts of milk from Milk-shop 3 and from Supermarket 3 differed significantly from each other, indicating that the origin and treatment of the milk is important in determining its quality.

Coliform counts in milk-shop milk ($n = 129$) varied greatly between milk-shops over the 6-week period, ranging from 0 to $3.4 \times 10^5$ coliforms per ml (Fig. 2), with 88 (68 %) samples having counts lower than 20 coliforms per ml, which is the maximum number allowed when the Petrifilm method of counting is used. The median value for milk-shop milk was 21 (77.8 %) E. coli positive. Unfortunately, on many of the plates containing 1 ml of undiluted milk from Milk-shop 4, it was impossible to accurately determine whether or not E. coli was present. These plates contained so many coliforms that all that could be observed were very large gas bubbles under the film. These were considered suspect samples. This is a drawback of the dry rehydrated film method for coliform and E. coli counts, since high coliform numbers obliterate E. coli organisms. Other methods such as the Modified Eijkman Test for E. coli, although more laborious and time-consuming to perform, might be more useful in such cases. Milk-shop 4 sold 14 (51.9 %) samples which were E. coli negative. The remaining thirteen (48.1 %) samples were either positive or suspected to be positive for E. coli. The high prevalence of E. coli in Milk-shops 1 and 4 is possible since the milk from these 2 milk-shops was not pasteurised correctly$^{21,32}$. Milk-shop 2 sold 1 sample that was positive for E. coli (Table 1), possibly indicating human contamination after pasteurisation by handlers who practice poor personal hygiene or by contact with water containing sewage. The national distributor’s milk was always negative for E. coli in 1 ml (Table 1). Fifty-four (40 %) of all milk-shop milk samples purchased contained the organism S. aureus in 1 ml (Table 1). One third

![Fig. 1: Standard aerobic colony counts of milk-shop milk and that of a national distributor.](image1)

![Fig. 2: Coliform counts of milk-shop milk and that of a national distributor.](image2)
of these organisms was found in correctly pasteurised milk and the other two-thirds in milk which was not correctly pasteurised. *S. aureus* in the latter group may have originated from animals with subclinical mastitis, as *S. aureus* is the dominant mastitis organism in South Africa, being prevalent in at least 75% of South African herds\(^5\). *S. aureus* in raw milk may also have originated from human carriers. Where the organism was isolated from milk which had been correctly pasteurised, it must have originated from the people who handle the milk, since this organism is destroyed by pasteurisation\(^5\). Surveys have shown that up to 60% of humans are nasal carriers of this organism, and that between 5% and 20% of people carry the organism as part of their normal skin flora\(^5\). The national distributor’s milk did not contain any *S. aureus* (Table 1).

If milk is not refrigerated, several strains of *S. aureus* can produce heat-stable enterotoxins that survive the pasteurisation process and cause food poisoning in man\(^11\). Of the 51 *S. aureus*-positive cultures which were tested for the production of enterotoxins, 4 (7.83%) produced heat-stable staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination of these. All the toxin producing strains isolated originated from Milkshop 2. *S. aureus* strains and SEA/SEB/SED by the other 2 strains. Bolstridge and Roth\(^7\) reported that 18.9% of *S. aureus* isolates from both raw and processed dairy products purchased in South Africa were found to be enterotoxigenic, with most producing enterotoxins A or C or a combination of A and C. Most food poisoning outbreaks involve enterotoxins A and D as they are produced under a much wider range of environmental conditions than B and C\(^7\). No *S. aureus* enterotoxin could be detected in 15 national-distributor milk samples tested. The production of enterotoxin by staphylococci can be completely managed by temperature control as multiplication of the bacteria and toxin formation are almost completely inhibited below 7°C\(^5\).

Of public health importance was the fact that 73 of 135 (54%) milk-shop milk samples purchased contained some type of inhibitory substances (Table 1). Residues are illegal in terms of the Act\(^7\). Since the milk was not analysed further to determine which substances were present they could consist of antibiotics or other antimicrobials such as formalin or hydrogen peroxide which may have been illegally added to the milk to increase the shelf-life. The results showed that the national distributor’s milk never contained any inhibitory substances (Table 1). The prevalence of inhibitory substances in milk-shop milk was high, ranging from 33.3% in Milkshop 5 to 92.6% in Milkshop 2. The Act\(^7\) states that milk should not contain any inflammatory product which may render the milk unfit for human consumption. Cows in very early or very late lactation, or cows with a low-grade or latent udder infection, are likely to produce milk containing an excessive number of somatic cells, consisting mainly of leucocytes and some epithelial cells\(^5\). Milk-shop milk somatic cell counts varied between 1.2 × 10⁶ and 1.6 × 10⁹ cells per ml, with a median count of 4.2 × 10⁶ cells (Fig. 3). Only 18.7% (25 of 135 samples) of somatic cell counts were above the legal limit of 500 000 cells/ml. The national distributor’s milk always had somatic cell counts of less than 150 000 cells per ml (Fig. 3) and differed significantly from all the milk-shops except for Milkshop 4. Somatic-cell counts are decreased in the clarifying process which is done at larger dairies and processing plants, and this may be the reason why the somatic cell count of the national distributor were so constant and so low over the 6-week period.

All milk samples tested by means of the brucella milk ring test (BMRT) were negative for antibodies to *Brucella abortus* which is a zoonosis and has not yet been eradicated from cattle in South Africa. Commercial pasteurisation effectively kills *B. abortus*\(^5\)\(^,\)\(^10\). As all milk samples were tested at least 2–3 times per week it is unlikely that there could have been false negatives. Seventeen *E. coli*-positive samples were further tested for the presence of *Salmonella* spp. in 1 ml, but these samples were all negative for the organism.

**CONCLUSIONS**

In conclusion, of the 135 pasteurised milk samples purchased from milk-shops, 117 (87%) were not fit for human consumption on the basis of all the criteria laid down in the Foodstuffs, Cosmetics and Disinfectants Act\(^7\). Milk-shop 1 never sold milk that was fit for human consumption, whereas the remaining 4 milk-shops, only complied with the Act between 4% and 33% of the time. All of the 79 samples purchased from a large national distributor passed all the criteria laid down in the Act.

The results showed that milk-shop milk differed significantly from the milk that originated from the national distributor and varied greatly between milk-shops and between sampling days over the 6-week period. Consumers are therefore unwittingly exposed to unnecessary health risks by drinking unsafe milk. These findings are similar to those found after a survey in South Africa in 1995 by the Department of Health\(^10\) which concluded that 73% of pasteurised milk samples did not comply with all the regulations. Their results included the milk of national distributors. In this study it was found that all the samples purchased from the national distributor consistently passed all the criteria laid down in the Act, and therefore samples that were obtained from national distributors in the national study may have improved the results to some extent.

The fact that nearly 40% of milk samples were most probably incorrectly pasteurised, and the high prevalence of *E. coli* and *S. aureus* in these raw milk samples proves...
the greater risk of raw milk. Susceptibility to food-borne pathogens varies greatly from person to person. High risk people who may be particularly susceptible to infection include immunocompromised people whose immune systems are deficient either because of an immunodeficiency disorder or because of treatment with immunosuppressive drugs. These would include pregnant women, transplant recipients, AIDS and cancer patients, very young infants, steroid users, and patients with chronic renal disease. South Africa has a high prevalence of HIV-positive people and milk-shop milk could be a real hazard to their health. Not only can unsafe milk affect the health of the consumer, but it may also have economic implications such as medical and hospitalisation costs, mortality costs, productivity losses, and the long-term reduction in quality of life. This could place a burden on primary health care services, the employers and employees due to absenteeism.

To produce safe, sound and wholesome milk for the consumer entails good production practices throughout the chain from the cow to the consumer. This includes the milking of healthy animals, the use of clean and hygienic equipment on the farm and during processing, maintenance of the cold chain throughout the production process, effective pasteurisation and prevention of post-pasteurisation contamination. People handling milk should be educated in safe food-handling techniques and proper personal hygiene practices including hand washing after using the lavatory. Training programmes for staff working in milk-shops is essential as these people work with food and are often ignorant of basic hygiene principles. Milk-shop owners (and dairy farmers) should institute hygiene programmes on the farm and in the shop that should consist of good manufacturing processes, quality control, hazard analysis and critical control point (HACCP) principles. There is also a need for more stringent control over milk-shops by the relevant authorities. Questions must be asked as to whether or not the local authority ever analysed the milk and if so, why they did not do anything about the results. A suggestion might be that people who work with perishable foods such as milk or meat that could affect the health of the consumer, would need to undergo some type of compulsory training before being able to work in a specific field, and that this training would include a component on the regulations concerning that industry as well as some knowledge of the processes involved. Public health aspects should also be part of the training. However, public education is also needed as legislation alone is insufficient.

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