Enumeration of potential microbiological hazards in milk from a marginal urban settlement in central South Africa

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Abstract

Milk samples from 60 randomly selected households in the Botshabelo township were collected and analysed for the presence of microorganisms. In a marked number of samples the total mesophilic, coliform and Escherichia coli counts exceeded the national standard (5000 cfu mL⁻¹ for total mesophilic counts; 10 cfu mL⁻¹ for coliforms and absence of E. coli in 1 mL for both raw and pasteurized milk intended for consumption). Both the total mesophilic and coliform counts exceeded the national standard in more than 80% of the samples and reached counts of up to 10¹⁰ and 10⁹ cfu mL⁻¹, respectively. The E. coli counts were between 0 and 10¹ cfu mL⁻¹ in 76.6% of the samples whilst counts of up to 10⁵ were found in some instances. Although specific national guidelines in terms of aerobic and anaerobic spore-forming organisms, members of the Enterobacteriaceae family, yeasts and moulds have not yet been set, the counts of these microbiota were also very high. The data suggested that the general food–hygiene-related knowledge and infrastructure of the community was lacking, affirming the relationship between socio-economic status and household hygiene. The elevated microbial counts resulting from ignorance towards proper handling and housekeeping practices, pointed to milk as a sure medium of food-related infection in the area.

Keywords: Milk; South Africa; Enumeration

1. Introduction

Being a major constituent of the diet, quality control of milk is considered essential to the health and welfare of a community. However, people from low-income groups have shown very little interest as to whether food and drink are good or detrimental to their health, their only concern being to buy enough food to keep them from starvation (Fox and Cameron, 1995). In developed societies, practices generally used to curb microbial proliferation in milk include pasteurization and refrigeration. These low-income groups, however, do not utilize such practices mainly due to lack of infrastructure and funds (Rohde, 1985; Collins et al., 1995). Adding to this problem, health and hygiene in South Africa have been characterized by a series of changes since a control body known as the Milk Board, acting as watchdog over the quality of milk, has recently been dismantled. This was done as part of the decentralization of the South African dairy industry in an attempt to promote free market trade (Coetzee, 1998). Since this dismantling a decline in the milk quality of informal milk suppliers, including street vendors in rural areas and informal settlements, has been observed whilst the milk quality in urban areas is, to a large extent, still in tact. A lack of knowledge concerning the dangers involved, the lack of basic infrastructure as well as poor housekeeping techniques were all thought to be possible causes for this decline in milk quality (Keller, 1995; Van der Westhuizen, 1999).

Being a nutritional, balanced foodstuff that contains few organisms when it leaves the udder, milk gets contaminated at various stages be it from the cow, milker (manual as well as automated), extraneous dirt or unclean process water (Stewart, 1978; Rohde, 1985; Banwart, 1989; Philips and Griffiths, 1990; Gruetzmacher and Bradley, 1999; Hayes et al., 2001). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable (Foster, 1990). Microbiota

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generally associated with milk and milk spoilage are coryneforms, micrococci and lactococci, and include genera such as *Pseudomonas*, *Brucella*, *Escherichia*, *Salmonella*, *Shigella* as well as *Bacillus* and *Clostridium* (Cousins and Bramley, 1981; Gilmour and Rowe, 1981; Dommett, 1992). Furthermore, the health hazards posed by milkborne zoonotic diseases like brucellosis, tuberculosis and mastitis-related enterotoxaemia are well described (Hobbs and Roberts, 1993).

Recent concerns voiced by the Environmental Health Sector of South Africa concerning proper household hygiene have been supported by reports that milk produced by some formal and increasing numbers of informal farmers often fail to meet the national standard (Keller, 1995). This emerging problem has been amplified by ignorance amongst the traditionally disadvantaged community regarding the necessity of milk pasteurization. It became necessary to assess the extent of milk hygiene in the mentioned informal settlements and investigate solutions in order to rectify these problem areas by identifying the factors that influence milk quality. The present study thus aimed to determine the hygienic conditions and possible sources of contamination associated with milk in a randomly selected area of a marginal urban settlement (the Botshabelo township (Section M) ca. 50km from the city of Bloemfontein in the Free State province) by the selective detection and enumeration of a variety of indicator organisms in the milk. An area was selected in the Botshabelo township that is relatively homogeneous with regard to infrastructure and general hygiene to conduct the research. Measures to address the prior hygienic status of milk were suggested.

2. Materials and methods

Previous studies in the area of Section M into the domestic practices (amongst others purchase, usage and storage) of milk have shown that 68.3% of residents purchased milk from formal bonafide supermarkets whilst the remainder (31.7%) obtained milk from tuck shops (informal, small scale general dealers and vendors) as well as from diverse sources such as local small-scale farmers and individuals selling milk from their homes as an extra income. In both the above cases 76.6% of the milk was purchased from bulk tanks, transported and stored in an open container. An alarming 80.7% of residents were unsure as to whether the milk they purchased was pasteurized (Van der Westhuizen, 1999).

Sixty households were randomly selected for this study. Sampling was performed early in the mornings from the milk containers used in the households on a weekly basis over a period of 6 weeks (10 households per week) with the aid of sterile sampling bags (Whirl-Pack, NASCO). The samples were kept on ice to restrict microbial multiplication and transported to the laboratory for analysis.

Serial dilutions were prepared with the use of Peptone buffer (Biolab, SA). The surface plating method (0.1 ml) was applied to quantify the various microbial groups (Herbert, 1990). For the enumeration of members of the family Enterobacteriaceae, violet red bile glucose agar (VRBG, Oxoid, SA) was used. The detection and enumeration of yeasts and moulds was done by means of acidified potato dextrose agar (PDA, Merck, SA) (Christen et al., 1993; Frank et al., 1993). Plate count agar (PCA, Merck, SA) was used to isolate aerobic mesophiles as well as the aerobic and anaerobic spore formers in the milk (Houghtby et al., 1993). Petrifilm™ (3M) was applied to enumerate the coliforms and *Escherichia coli* (Matter et al., 1990). To enumerate the spores of spore-forming organisms, the samples were exposed to 80°C for 10 min to destroy vegetative cells and transferred to the PCA plates. The anaerobic spore-forming organisms were incubated in anaerobic flasks with Anaerocult A and Anaerotest (Merck, SA) to confirm an anaerobic atmosphere.

The aerobic spore-forming organisms and the PDA plates were incubated for 48 h at 25°C, while the Petrifilm™, PCA plates, VRBG agar plates and the anaerobic spore-forming PCA plates were incubated at 35°C for a period of 48 h. A colony counter (Gerber) was used for enumeration. Micro-organisms belonging to the family Enterobacteriaceae produced round, purple colonies, that was 1–2 mm in diameter and surrounded by purple haloes (Mossel et al., 1986). *Escherichia coli* was characterized by blue colonies associated with gas bubbles, whereas coliforms were characterized by red colonies with gas bubbles. All counts were reported as presumptive because the methodologies used were stipulated in national legislation as being adequate for the assessment of conformance (South Africa, 1997).

3. Results

3.1. Patterns and numbers of microbial groups

The distribution of the various microbial groups found in milk from the Botshabelo township is shown in Fig. 1. The total aerobic mesophilic counts (Fig. 1A) varied considerably from one household to another with counts as high as $10^{10}$ cfu ml$^{-1}$ in a number of cases. With the exception of two households, all the samples exceeded the national standard of 50 000 cfu ml$^{-1}$. The results further showed that the majority of households had a total mesophilic count in the range $10^9$–$10^{10}$ cfu ml$^{-1}$ (28.3%) with counts in 16.6% of the households as high as $10^7$–$10^{10}$ cfu ml$^{-1}$. The coliforms
showed a more widespread distribution amongst households (Fig. 1B) with numbers ranging from $10^2$ to $10^9$ cfu ml$^{-1}$ and were, in general, found in high quantities (40% in the $10^2$–$10^6$ cfu ml$^{-1}$ and 26.6% in the $10^7$–$10^8$ cfu ml$^{-1}$ intervals). Similar to Fig. 1A that shows the national standard for total mesophilic counts, Fig. 1B indicates the coliform counts compared with the national standard ($10^1$ cfu ml$^{-1}$) for both pasteurized and raw milk intended for consumption. All samples exceeded this standard without exception.

A 23.3% incidence of *E. coli* in the range of $10^4$–$10^6$ cfu ml$^{-1}$ was found whereas the organisms were
not detected in the remaining samples (Fig. 1C). Thus, a percentage of 76.7 of samples conformed to the national standard for \( E. coli \), which is 0 per 1ml. The Enterobacteriaceae counts varied considerably amongst the various households reaching levels of \( 10^{5} \text{cfu ml}^{-1} \). The highest occurrence was \( 10^{6} \) and \( 10^{7} \text{cfu ml}^{-1} \) (33.3%) followed by \( 10^{5} \) and \( 10^{6} \text{cfu ml}^{-1} \) (26.6%, Fig. 1D).

Comparing the counts of the aerobic spore-forming bacteria (Fig. 1E) with the anaerobic spore-forming bacteria (Fig. 1F), more variation occurred in the latter. Quantities of 13.3% (aerobic) and 43.3% (anaerobic) occurred in the \( 10^{0}–10^{1} \text{cfu ml}^{-1} \) range. Notable were the relatively high counts in the range of \( 10^{2}–10^{3} \text{cfu ml}^{-1} \) (73.3% for aerobic and 48.3% for anaerobic spore-forming organisms). The yeasts (Fig. 1G) showed higher counts (70%) in the \( 10^{0}–10^{1} \text{cfu ml}^{-1} \) range than the moulds (46.6%; Fig. 1H). In the higher ranges the yeasts generally had higher counts than the moulds, especially in the \( 10^{3}–10^{4} \) and \( 10^{5}–10^{7} \text{cfu ml}^{-1} \) intervals. In general, the moulds appeared stratified whereas the yeasts were widely dispersed amongst the households.

3.2. Average counts

The composition of the microbial population generally inhabiting milk in the township, as represented by the mean counts, is shown in Fig. 2. The mean count for total aerobic mesophilic organisms was \( 8.6 \times 10^{8} \text{cfu ml}^{-1} \) (\( \pm 1.1 \times 10^{9} \text{cfu ml}^{-1} \)), coliforms \( 6.7 \times 10^{7} \text{cfu ml}^{-1} \) (\( \pm 1.7 \times 10^{8} \text{cfu ml}^{-1} \)), \( E. coli \) \( 1.2 \times 10^{4} \text{cfu ml}^{-1} \) (\( \pm 3.1 \times 10^{4} \text{cfu ml}^{-1} \)) and Enterobacteriaceae \( 2 \times 10^{2} \text{cfu ml}^{-1} \) (\( \pm 8.6 \times 10^{2} \text{cfu ml}^{-1} \)). Mean counts for the aerobic and anaerobic spore-forming organisms were \( 4.7 \times 10^{2} \text{cfu ml}^{-1} \) (\( \pm 5.8 \times 10^{2} \text{cfu ml}^{-1} \)) and \( 2 \times 10^{3} \text{cfu ml}^{-1} \) (\( \pm 7.4 \times 10^{3} \text{cfu ml}^{-1} \)), respectively. The yeasts showed an average of \( 2.3 \times 10^{6} \text{cfu ml}^{-1} \) (\( \pm 9.7 \times 10^{6} \text{cfu ml}^{-1} \)) in contrast with the \( 1.1 \times 10^{3} \text{cfu ml}^{-1} \) (\( \pm 3.8 \times 10^{3} \text{cfu ml}^{-1} \)) count for moulds (Fig. 2).

4. Discussion

Because \( E. coli \) is generally regarded as an indicator of faecal contamination, it can be concluded that the samples were contaminated with faecal material to a considerable extent. \( Escherichia coli \) is, furthermore, a known causative agent of diarrhoea and other food-borne-related illnesses through the ingestion of contaminated foodstuffs. Pathogenic members of the coliform group as well as the Enterobacteriaceae family are represented by genera such as \( Salmonella \) and \( Shigella \) and are found in the intestines of humans and animals (Le Minor, 1984; Rowe and Gross, 1984; Collins et al., 1995; Hayes et al., 2001). With these organisms well represented in the samples the risk of a variety of gastro-enteritic illnesses and enterotoxaemia is eminent (Maurelli and Lampel, 1994; Jay, 2000). The distribution of total mesophilic counts, coliforms and \( E. coli \) (required by National Legislation to indicate general and faecal contamination) across the various intervals suggest that there does not necessarily exist a relationship between the total mesophilic population and coliforms (\( r^2 = -0.04 \)) and \( E. coli \) (\( r^2 = -0.017 \)) in heavily contaminated milk. A weak negative correlation

![Fig. 2. The average occurrence of bacterial populations in milk from M Section region: Botshabelo, Free State.](image-url)
of $r^2 = -0.15$ was furthermore found between $E. \text{coli}$ and coliforms. The source of contamination, whether it is from faecal or other origin, therefore plays a key role in the prevalence of the various populations in the milk.

Although it was not known whether the milk was pasteurized or not because source of milk are so many, from such a variety of sources, the high numbers of aerobic and anaerobic spore-forming organisms may be indicative of the degree, or lack of, pasteurization (Anderson et al., 1995). A number of spore-forming bacteria are known to, apart from causing spoilage and producing off-flavours, cause food-poisoning by producing heat labile enterotoxins (Eley, 1992; Reed, 1994; Anderson et al., 1995; Hittu and Punj, 1999). Although high counts of yeasts and moulds were found, their growth in milk is rather uncommon, as the pH of milk is neutral, causing bacteria to predominate (Frazier and Westhoff, 1988; Pitt and Hocking, 1997). The undesirability of excessive amounts of fungi lies in their ability to sensorially degrade milk (Banwart, 1989; Jacob, 1989). The most common mould found in milk, *Geotrichum candidum*, have been reported to proliferate in milk due to unclean processing lines and unhygienic handling, whereas the growth of the yeast *Cladosporium butyri* in milk is considered undesirable due to its ability to cause rancidity (Pitt and Hocking, 1997).

Based on the exceedingly high microbial counts found in this study, it can be concluded that the milk poses a definite health risk in the study area. Non-conformance to the National Standard is, however, not necessarily indicative of the presence of pathogens but rather a measure of the general hygiene. This is especially applicable keeping in mind that the South African legislation compares favourably with the US Public Health Service recommendations of 1965 (Total bacteria not exceeding $100000 \text{ cfu ml}^{-1}$ (commingled) and $300000 \text{ cfu ml}^{-1}$ (non-commingled) for Grade A milk for pasteurization; total bacteria not exceeding 20000 cfu ml$^{-1}$ and coliforms 10 cfu ml$^{-1}$ for Grade A pasteurized milk) (Potter and Hotchkiss, 1995; Jay, 2000). It is likely that the high counts for the various micro-organisms can be attributed to: (1) ignorance of the residents towards the fundamental aspects of good and safe housekeeping; (2) lack of basic infrastructure for storage and handling; and (3) milk of inferior quality purchased from suppliers. It should be pointed out, however, that in this study the milk from the suppliers was not sampled separately but only after being handled and stored by the consumers. Lack of electricity, lack of proper sanitation and inadequate water supply may further bring about the proliferation of contaminants in the milk. More research regarding the milk quality and milking protocols of small-scale farmers in the Botshabelo area, supplying milk to the local vendors and general dealers, should be conducted. In similar areas, descriptive research regarding the exact usage and storage practices of milk in particular, is necessary.

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**References**


