# AN ASSESSMENT OF THE BACTERIOLOGICAL QUALITY OF COW MILK PRODUCTS SOLD IN KEFFI METROPOLIS, NASARAWA STATE, NIGERIA

**M. D. Makut* and J. Ishaya**

Microbiology Unit, Department of Biological Sciences
Nasarawa State University, PMB 1028, Keffi, Nigeria
*****Correspondence author: makmakwin@yahoo.com

**Received:** June 20, 2012; **Accepted:** 07, 2012

## Abstract

*Samples of raw cow milk ('Madara') and its products, skimmed milk ('Nunu'), local yoghurt ('Kindirmo') and milk fat ('Mai shanu') were collected in triplicates from 10 different locations in Keffi Metropolis, and were analyzed for their bacteriological quality. Total bacterial counts were also obtained using plates in triplicates and these ranged from 2.3 x $10^3$ – 5.5 x $10^3$ for milk fat ('Mai shanu') and 3.2 x $10^3$ – 3.8 x $10^3$ for raw milk ('Madara'). Staphylococcus aureus, Escherichia coli, Lactobacillus spp, Salmonella spp, Streptococcus spp. and Vibrio spp were isolated from Skimmed milk, while Lactobacillus spp., Staphylococcus aureus and Escherichia coli were isolated from Local yoghurt. Salmonella spp, Staphylococcus aureus and Escherichia coli were isolated from milk fat, whereas only Staphylococcus aureus and Escherichia coli were found in raw milk. The presence of Staph. aureus, E. coli, Vibrio spp, Salmonella and Streptococcus in the raw milk and all its products sold in Keffi has serious health implication to the consumers of these products, since strains of most species of these organisms are pathogenic to humans.*

***Keywords**: Bacteriological quality, cow milk, Keffi, Nigeria.*

## INTRODUCTION

Milk has been defined as the normal secretion of the mammary gland of mammals, and has also been described as a natural food with a clear nutritional value (Aumaitre, 1999). The principal components of milk are water, fat, protein and lactose. The fatty acids present in fresh milk are mainly in the form of fat globules which are surrounded by phospholipid rich layer known as the milk fat globule membrane (Adams & Moses, 1995). The components of the milk provide appropriate physical and chemical properties which create a favourable medium for the multiplication of microorganism (Poelma *et al*., 2001). Milk from its natural source is a sterile product (Frazier & Westhoff, 1978), but its rich nutritional composition makes it favourable for microbial habitation. Douglas (1995) reported that milk is sterile at secretion in the udder, but contamination may occur at the time it leaves the udder. He further reported that except in the case of mastitis, bacteria at this point are harmless and few in number and that further contamination of the milk by microorganisms can take place during milking, handling, storage and other pre–processing activities. During the normal milking operation, milk is subjected to contamination from the animal especially the exterior of the udder and adjacent areas (Frazier & Westhoff, 1978). Douglas (1995) reported that lactic acid bacteria, coliforms, spoilage and pathogenic bacteria are often associated with milk after milking. The main sources that contribute to these microorganisms in milk are the udder interior, the teat exterior, the immediate surroundings, milking and milk handling equipment and also milk contacts surfaces (Adams & Moss, 1995). If dairy utensils or the milk contact surfaces are inadequately cleaned, sanitized, and dried, bacteria may develop in large numbers in the dilute, milk–like residue and enter the next milk. Undesirable bacteria from these sources include lactic acid bacteria, streptococci, coliforms, psychro–trophic gram–negative rods and thermodurics (Frazier & Westhoff, 1978). Milk contact surfaces add few bacteria per milliliter of milk, but under very poor sanitary conditions these sources may increase the bacterial content of the milk by several millions per milliliter. Other possible sources of contamination are the hands and arm of the milker or dairy workers, the air of the barn or milking parlor, and the utensils (Frazier & Westhoff, 1978). The quality of the water supply used in the milking parlor for cleaning and rinsing the cows will have some effect on the quality of the milk. Bacteria that get onto the outside of the teat may be able to invade the opening and hence the udder interior (Parihar & Parihar, 2008).



holders and storage tanks are the principal sources of the microorganisms found in raw milk (Adams & Moss, 1995). Milk is a nutritious medium and if equipment is poorly cleaned, milk residues on surfaces that are frequently left wet will act as a focus for microbial growth which can contaminate subsequent batches of milk (Adams & Moss, 1995). Nebedum & Obiakor (2006) reported that contamination may come from external sources like air, soil, water, milking utensils and personnel, as well as the disease pathogens of the animals.

The lactic acid bacteria such as *Lactococcus lactis (Streptococcus lactis), Lactococcus cremoris (Streptococcus cremoris), Lactobacillus casei, Lactobacillus lactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophiles* and *Leuconstoc* species are normally present in the milk, and are able to ferment lactose to lactic acid (Gaman & Sherrington, 1965; Frazier & Westhoff, 1978; Douglas, 1995) The lactic acid bacteria are usually used as starter cultures in the production of cultured dairy products such as yogurt. Some of these lactic acid bacteria can also be shedded from the udder surface (Desmasures *et al*., 1997) or generally from the body of the cows (Salama *et al.,* 1995). Therefore, an inadequate hygiene around the udder would preserve this type of flora (Bacic *et al.,* 1968).Coliforms can also cause rapid spoilage of milk because their ability to degrade milk protein and to ferment lactose with the production of acid and gas. Microbial spoilage of milk can also occur by the activities of such organisms like *Pseudomonas fluorescence* and *Pseudomonas fragi*, which often result in deterioration of the milk texture, color, odour or flavour due to break down of protein, carbohydrates and fats by these bacteria (Douglas, 1995; Parihar & Parihar, 2008). Pathogenic microorganisms commonly associated with cow milk include *Bacillus cereus,* which causes food poisoning, *Listeria monocytogenes*, which causes listeriosis, *Yersinia entercolitica*, which causes gastroenteritis, *Salmonella* spp, which causes typhoid fever, *Escherichia coli* 0157 H, which causes travelers' diarrhea, and *Campylobacter jejuni*, which causes enteritis (Frazier & Westhoff, 1978; Douglas, 2005).

Poelma *et al*. (2001) reported that milk has natural bacteriostatic properties and this is due to the presence of a number of antimicrobials which are present at very low concentrations in the cow milk, and these remain effective for several hours after milking. Faye & Loiseau (2002) reported that the processing of cow milk is usually faced with many problems related to standards and quality, which are often difficult to meet. Many factors contribute to this phenomenon, and these include the udder interior, teat exterior, immediate surroundings, geographical area, breeding, climate conditions, milking technique, milking equipments and nutritional factors present in the milk (Collins, 1976; Nebedum & Obiakor, 2006). Furthermore, Aumaitre (1999) reported that inadequate sanitation during milking and handling constitute an additional factor that can cause deterioration of milk and milk products, and cooling to low temperature reduces the bacterial growth, but cannot eliminate the microorganisms already present in the milk.This favours the preponderance of psychrotrophic bacteria in milk and dairy products (Cousin, 1982), which are also proteolytic agents that lead to deterioration of milk and milk products. In cooled milk, pathogenic bacteria can persist, as can deterioration bacteria, saprophytic bacteria and yeast. Some of the pathogens can be dangerous to humans and can trigger non–specific digestive disorders or specific diseases (Faye & Loiseau, 2002). Frazier & Westhoff (1978) reported that fewer organisms enter the milk when a milking machine is used rather than when milking is by hand, and such contamination is reduced by clipping the cow especially the flanks and udder, grooming the cow and washing the udder with water or a germicidal solution before milking. Contamination of the cow with soil, water and manure is reduced by paving and draining barnyards, keeping cows from stagnant pools and cleaning manure from the barns or milking parlors.

Most of the organisms involved in the fermentation process are usually of three main groups, bacteria, yeast and mould. Of these lactobacilli (*L. acidophilus* and *L. bulgaricus*), lactococci (*Lactococcus cremoni* and *Lactococcus lactis*), *Streptococcus thermophilus, Leuconostos spp*, and *Saccharomyces* species are the most prominent, and each of these gives milk products characteristic flavour. Sour milk is thus not a uniform product. Variations in milk composition are often due to bacterial flora and ambient temperatures which give rise to products of varying qualities (Gaman & Sherrington, 1965; O' Connor & Tripachi, 1995). The presence of certain strains of microorganisms such as lactobacilli and *Streptococcus* sp may be beneficial (Perdigon *et al.,* 1987). Several of these have been found to have probiotic properties and immunomodulatory function. Several strains of lactobacilli have been reported to display stimulatory properties on cells of innate immunity i*n vitro* and *in vivo* in both animal models and in humans fed with fermented milk products containing probiotics (Ibnou–Zekni *et al.,* 2003). These effects were however found to be species and strain specific, since not all strain of any particular microorganisms have equal probiotic efficacy (Perdigon *et al*., 1987; Schifrin *et al*., 1995). Probiotic properties generally inhibit the growth of harmful bacteria and prevent the production of harmful metabolites (Bowley, 2005). In milk, other microorganisms such as psychrotrophic organisms are principally involved in spoilage. Most psychrotrophs are destroyed by pasteurization temperature. However, some like *Pseudomonas fluorescence* and *Pseudomonas fragi* can produce proteolytic and lipolytic extracellular enzymes which are heat stable and capable of causing spoilage (Douglas, 1995). Some species and strains of *Bacillus, Clostridium, Corynebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus* and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problem (Douglas, 1995). Information on the microbial content of milk can be used to determine its sanitary quality and production conditions (Frazier & Westhoff, 1978).

This investigation aimed at determining the bacterial flora of cow milk and milk products sold in Keffi metropolis with a view to ascertaining the bacteriological quality of these products for human consumption.

## MATERIALS AND METHODS

### Study Area

The study was carried out in Keffi town, where the cow milk products are sold. Keffi is about 68km away from Abuja, the Federal Capital, and is located on latitude 8°5'N and longitude 7°50'E, and an altitude of 85 meters above sea level. It is situated North–West of Lafia, the Capital city of Nasarawa State (Akwa *et al.*, 2007)

### Sample Collection

Samples of raw milk (Madara), skimmed milk (Nunu), local yoghurt (Kindirmo) and milk fat (Mai Shanu) were each collected at random from ten different locations. Twenty milliliters (20 ml) of each sample was collected into a sterile bijou bottle and placed into a cooler containing ice block which helped to maintain low temperature. The samples were immediately taken to the laboratory for analysis. The 10 different locations where samples were collected included Dadin Kowa, Makera, Angwan Waje, Main Market, Angwan Lambu, High Court, Government Reserve Area (G.R.A), Emir's Palace, Kofar Hausa and Tudun Amama.

### Isolation and Identificatiom of Bacteria

Microorganisms were isolated from samples using the pour plate method of Collins & Lyne (2004). Small amount (1.0 ml) of each sample was initially diluted with 9.0ml of sterile distilled water, which was serially diluted. Inocula were aseptically obtained from $10^{-3}$ dilution and mixed with molten agar which was then poured into Petri dishes, respectively. Plates were incubated in triplicates at the temperature 37°C for 24 hours. The media used were MacConkey Agar (MA), Salmonella–Shigella Agar (SSA), Mannitol Salt Agar (MSA), Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB) and De Man Rogosa Sharpe Agar (MRS). Total Aerobic Plate Count of each sample from each location was determined by counting the total number of colonies on Nutrient Agar. The bacterial isolates were identified using cultural, microscopic (morphological) and biochemical characteristics (Collins & Lyne, 2004). The identification was carried out with reference to Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbon, 1974).

### Statistical Analyses

The data obtained were subjected to analyses using means, mean deviation, Two–Analysis of Variance (ANOVA) and test for Least Significant Difference (LSD).

## RESULTS AND DISCUSSION

The Microbial Load (Total Aerobic Plate Counts) of the skimmed milk ('Nunu'), local yoghurt ('Kindirimo'), milk fat ('Mai shanu') and raw milk ('Madara') sold at ten different locations of Keffi metropolis are presented in Tabl 1, while Table 2 shows the counts of bacterial isolates recorded in the four milk products.

The results further reveal that there was significant difference ($P < 0.05$) in the microbial counts of the four different milk products sold in Keffi, but these counts did not vary with locations. However, Table 1 reveal that Skimmed milk ('Nunu') had the highest level of microbial load with an average count of 5.10 x $10^3$ cfu/ml, followed by Milk fat ('Mai shanu') which had 4.59 x $10^3$ cfu/ml, Raw milk ('Madara') which had microbial count of 3.19 x $10^3$ cfu/ml, and local yoghurt ('Kindirmo') which had the least microbial load of 2.85 x $10^3$ cfu/ml. The result of Table 2 shows that *Staphylococcus aureus* and *Escherichia coli* were present in all the milk products sold in Keffi metropolis. Milk fat ('Mai Shanu') had the highest count (3.5 x $10^3$ cfu/ml) of *Staphylococcus aureus*, followed respectively by yhe counts of raw milk (3.2 x $10^3$ cfu/ml), Local yoghurt (2.9 x $10^3$ cfu/ml) and Skimmed milk (2.8 x $10^3$ cfu/ml). However, Skimmed milk had the highest count (7.7 x $10^3$ cfu/ml) of *Escherichia coli*,which was followed by Milk fat (5.5 x$10^3$ cfu/ml), Raw milk (3.5 x $10^3$ cfu/ml) and Local yoghurt (2.3 x $10^3$ cfu/ml), respectively. *Lactobacillus* spp. which were only present in Skimmed milk and Local yoghurt with counts of 5.3 x $10^3$ cfu/ml and 4.8 x $10^3$ cfu/ml, respectively. *Salmonella* spp. were found to be present in Skimmed milk and Milk fat with counts of 3.2 x$10^3$ cfu/ml and 2.4 x $10^3$ cfu/ml, respectively. *Sreptococcus* spp. and *Vibrio* spp. were found in Skimmed milk with counts of 3.1 x $10^3$ cfu/ml and 2.3 x$10^3$ cfu/ml, respectively.

**Table 1: Total Aerobic Plate Count (cfu/ml) of Microorganisms from Cow Milk Products Sold at the Different Locations in Keffi**

| Locations | Total Aerobic Count (cfu/ml) of Cow milk products | | | |
|---|---|---|---|---|
| | **Skimmed milk** | **Local yoghurt** | **Milk fat** | **Raw milk** |
| Angwan Lambu | $3.2 \times 10^3 \pm 0.1$ | $3.0 \times 10^3 \pm 0.0$ | $3.7 \times 10^3 \pm 0.1$ | $2.2 \times 10^3 \pm 0.0$ |
| Angwan Waje | $4.2 \times 10^3 \pm 0.1$ | $3.0 \times 10^3 \pm 0.0$ | $3.5 \times 10^3 \pm 0.1$ | $2.7 \times 10^3 \pm 0.0$ |
| Dadin Kowa | $5.4 \times 10^3 \pm 0.1$ | $2.1 \times 10^3 \pm 0.1$ | $2.9 \times 10^3 \pm 0.2$ | $3.4 \times 10^3 \pm 0.1$ |
| Emir's Palace | $6.8 \times 10^3 \pm 0.1$ | $2.6 \times 10^3 \pm 0.1$ | $3.1 \times 10^3 \pm 0.1$ | $3.5 \times 10^3 \pm 0.1$ |
| G R A | $4.9 \times 10^3 \pm 0.0$ | $2.5 \times 10^3 \pm 0.1$ | $3.9 \times 10^3 \pm 0.1$ | $3.8 \times 10^3 \pm 0.1$ |
| High Court | $7.2 \times 10^3 \pm 0.0$ | $3.8 \times 10^3 \pm 0.1$ | $6.6 \times 10^3 \pm 0.0$ | $3.6 \times 10^3 \pm 0.0$ |
| Kofar Hausa | $4.5 \times 10^3 \pm 0.1$ | $2.9 \times 10^3 \pm 0.0$ | $7.0 \times 10^3 \pm 0.0$ | $2.7 \times 10^3 \pm 0.1$ |
| Main Market | $7.8 \times 10^3 \pm 0.1$ | $3.5 \times 10^3 \pm 0.1$ | $3.6 \times 10^3 \pm 0.0$ | $3.0 \times 10^3 \pm 0.1$ |
| Makera | $2.0 \times 10^3 \pm 0.0$ | $2.3 \times 10^3 \pm 0.0$ | $4.3 \times 10^3 \pm 0.1$ | $3.2 \times 10^3 \pm 0.0$ |
| Tudun Amama | $5.0 \times 10^3 \pm 0.1$ | $2.8 \times 10^3 \pm 0.0$ | $7.3 \times 10^3 \pm 0.0$ | $3.8 \times 10^3 \pm 0.1$ |
| **MeanLSD(P = 0.05)** | **$5.10^a$** | **$2.85^c$** | **$4.59^{a\,b}$** | **$3.19^{b\,c}$** |

**Table 2: Bacterial Counts of Isolates from Cow Milk Products Sold in Keffi Metropolis**

| Isolates | Counts (cfu/ml) | | | |
|---|---|---|---|---|
| | **Skimmed Milk** | **Local Yoghurt** | **Milk Fat** | **Raw Milk** |
| *Staphylococcus aureus* | $2.8 \times 10^3 \pm 0.1$ | $2.9 \times 10^3 \pm 0.0$ | $3.5 \times 10^3 \pm 0.0$ | $3.2 \times 10^3 \pm 0.0$ |
| *Escherichia coli* | $7.7 \times 10^3 \pm 1.3$ | $2.3 \times 10^3 \pm 0.0$ | $5.5 \times 10^3 \pm 0.1$ | $3.5 \times 10^3 \pm 0.0$ |
| *Lactobacillus* spp. | $5.3 \times 10^3 \pm 0.0$ | $4.8 \times 10^3 \pm 0.2$ | $0.0 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ |
| *Salmonella* spp. | $3.2 \times 10^3 \pm 0.1$ | $0.0 \times 10^3 \pm 0.0$ | $2.4 \times 10^3 \pm 0.1$ | $0.0 \times 10^3 \pm 0.0$ |
| *Streptococcus* spp. | $3.1 \times 10^3 \pm 0.1$ | $0.0 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ |
| *Vibrio* spp. | $2.3 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ | $0.0 \times 10^3 \pm 0.0$ |

The presence of *Staphylococcus aureus* and *Escherichia coli* in all the cow milk products analyzed revealed that the four milk products sold in Keffi are contaminated and this poses a danger to the consumers of these products. *Staphylococcus aureus* is associated with a number of human infections including staphylococcal food poisoning. The presence of *Staphylococcus aureus* in the milk and milk products may have arisen from the human handlers or even from diseased cows infected with mastitis, whose causative agent is *Staphylococcus aureus* (Frazier & Westhoff, 1978). *Escherichia coli* is a bacterium of mammalian enteric origin which implies that all the milk products are contaminated with contaminants of feacal origin, and therefore not fit for consumption. Moreover, certain strains of *Escherichia coli* such as *E. coli* 015 H are known to cause Traveler's diarrhea. *Escherichia coli* in these the milk and milk products may have come from contamination with feaces of the cows since these animals are known to sleep on its feaces. However, the existence of *E. coli* in all the samples may not necessarily indicate a direct fecal contamination of the milk and milk products, but may indicate poor hygiene and sanitary practices during milking and further handling by the human handlers of these products (Faye & Loiseau, 2002; Nebedum & Obiakor, 2006).

Skimmed milk (Nunu) was the most heavily contaminated with an average microbial load of 5.10 x $10^3$ cfu/ml, and had all the different species of bacteria that were isolated which included *Staphylococcus aureus, Escherichia coli, Lactobacillus* spp., *Salmonella* spp., *Streptococus* spp. and *Vibrio* spp. Milk fat (Mai Shanu) was the second most contaminated milk product. In addition to the presence of *Staphylococcus aureus* and *Escherichia coli*, there was also the presence of *Salmonella* spp in the Milk fat. Local yoghurt had the least microbial count of 2.85 x $10^3$ cfu/ml, even though there was presence of *Lactobacillus* spp in addition to *Staphylococcus aureus* and *Escherichia coli* isolated. *Salmonella* spp, *Streptococcus* spp and *Vibrio* spp isolated were not only contaminants, but are known to cause various infections in humans. These pathogenic organisms may have entered the milk and milk products from the water sources used in the cleaning or washing of the utensils/containers for milk collection and processing, or even from the body and hands of the human handlers of these products (Adams & Moss, 1995). The presence of *Lactobacillus* spp in Skimmed milk and Local yoghurt was indicative of the role these species play in the fermentation of milk leading to production of yoghurt. It has been reported that *Lactobacillus bulgaricus* is used industrially as a starter culture that gives yoghurt its desirable taste and flavour (Gaman & Sherrington, 1965; O'Connor & Tripachi, 1995)). This therefore suggests that the taste andflavour of the skimmed milk and local yoghurt must have been produced by the *Lactobacillus* spp present in these products. Perdigon *et al*. (1987) also reported that the presence of certain strains of microorganisms such as lactobacilli and certain species of *Streptococcus* in milk and milk products may be beneficial. Several of these bacteria have been found to have probiotic properties and immunomodulatory function. Several strains of *Lactobacillus* have been reported to display stimulatory properties on cells of innate immunity *in vitro* and *in vivo* in both animal models and humans that are fed with fermented milk products containing probiotics. These effects were however found to be species and strain specific since not all strains of any particular microorganisms have equal probiotic efficacy (Perdigon *et al*., 1987; Schiffrin *et al.,* 1995). Probiotics are also known to inhibit the growth of harmful bacteria and prevent the production of harmful metabolites (Bowley, 2005).

The presence of *Staphylococcus aureus, Escherichia coli, Salmonella* spp, *Sterptococcus* spp and *Vibrio* spp in the milk and milk products sold in Keffi metropolis is of serious health implication to the consumers of these products. The National Agency For Food, Drug Administration and Control (NAFDAC) is concerned with setting standards for food, and such standards state that the count for any pathogen like *Salmonella* or *E. coli* on inoculated plates should be zero colony forming units per milliliter (0 cfu/ml), which means that no colony should be found in plates inoculated with food samples for such food to considered acceptable for human consumption. This implies that the cow milk and milk products sold in Keffi during this investigation did not meet the acceptable standard, and therefore they were unwholesome for human consumption. Parihar & Parihar (2008) recommended a number of measures which can be taken to minimize milk contamination from udder exterior which would certainly improve the microbiological quality of the processed cow milk. These measures include providing enough clean bedding and regular replacement of the bedding materials for milk producing cows, removal of slurry (faeces and urine) from bedding areas at least twice daily, shaving of udders and trimming of cow tails, washing of teats with warm water containing disinfectant and drying such teats singly with paper towels, keeping the milking parlour floor clean during milking, and thorough cleaning of teat cups if they fall off during milking.

Recommendations are also neccessary for milk handlers and salers in order to ensure that milk of

acceptable quality is milk processed and sold to consumers. There is need for the milk handlers to maintenance of personal hygiene, and to use clean and sterilized utensils during milking and processing of the milk and milk products. The use of clean portable water for washing of utensils, processing of the milk and milk products, cannot be over–emphasized as a way of minimizing contamination of milk sold to the public by the local milk salers.

## CONCLUSION

The microbial load of the various milk and milk products sold in Keffi metropolis differ significantly ($P < 0.05$). The level of microbial contamination varied in the different milk and milk products. Skimmed milk (Nunu) was found to be the most heavily contaminated, followed by milk fat (Mai Shanu), local yoghurt was the least contaminated. However, the microbial load of the various milk and milk products in the different locations of the Keffi metropolis were found to be similar ($P > 0.05$).

## ACKNOWLEDGEMENTS

The authors are grateful to the Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria, for providing the laboratory items used in this investigation.

## REFERENCES

Akwa, V. L., Binbol, N. L., Samaila, K. L. & Marcus N. D. (2007). Geographical Perspective of Nasarawa State. Onaivi printing and Publishing Company Ltd. Keffi, Nigeria, p. 3.

Aumaitre, A. (1999). Quality and safety of animal products. Livestock Production Science, 59: 113–124.

Adams, M. R. & Moss, M. O. (1995). Food Microbiology. A New Age International Publisher Limited, New Delhi.

Bacic, B., Jackson, H. & Clegg, L. (1968). Distribution of bacteria in milk drawn from the cow's udder. J. Dairy Sci. 51, 47–49.

Bowley, A. (2005). Vignette: Probiotics. Nutriview, 1: 8.

Buchanan, R. F. & Gibbon, N. E. (1974). Bergey's Manual of Determinative Bacteriology. Cambridge University Press, London.

Collins, C. H. and Lyne, P.M. (2004). Identification and Cultural Method in Microbial Methods (8th Edn). Butterwort, London.

Collins, E. B. (1976). Use of preservative in milk and dairy products. J. Dairy Sci., 50: 599.

Cousin, M. A. (1982). Importance in milk and dairy product. J. Food Protection, 45: 172–207.

Douglas, G. (1995). Dairy Science and Technology Education, University of Guelph, Canada.

Desmasures, N., Opportune, W. & Gueguen, M. (1997). *Lactococcus* sp.,Yeast and *Pseudomonas* sp. on teats and udder of milking cows as potential sources of milk contamination. Int. Diary J. 7: 643–646.

Faye, B. & Loiseau, G. (2002). Sources of Contamination in Dairy Supply Chain and Approaches to Quality Control. Advanced Method for Innovation in Science Department, pp. 8–13.

Frazier, W. C. & Westhoff, D. C. (1978). Food Microbiology (3rd edition) Tata McGraw–Hill Publishing Company Limited, New Delhi.

Gaman, P. G. & Sherrington, K. B. (1965). *Yoghurt*: The Science of Food. Pergamon press, London.

Ibnou–Zekni, N., Schiffrin, E. J. & Vonder W. T. (2003). Divergent patterns, of colonization and immune response elicited from two intestinal *Lactobacillus* strains that display similar properties *in vitro* infect. Immunity, 71, 428–436.

Nebedum, J.O. and Obiakor, T. (2006). The effects of different preservation methods on the quality of Nunu, a locally fermented Nigerian dairy product. Afr. J. Biotechnol, 6: 454– 458.

O'Connor, C. B. & Tripachi, B. R. (1995). Milk Processing Technique–Fermented Milk in Rural Dairy Processing Training Series: Module, 3: 53–54.

Parihar, P. & Parihar, L. (2008). Dairy Microbiology. Agrobios, India. 428p.

Perdigon, G., Alvarez, S., Oliver, G. & Gobbato, N. (1987). Immune system stimulation by probiotics. J. Dairy Sci., 78: 1597–1606.

Poelma, P. L., Andrews, W. H. & Siviker, J. H. (2001). Salmonella In: F.P Downes (ed). Compendium of Method for the Microbiological Examination of Food. American Public Health Association, Washinton DC, pp. 286–320.

Salama, M. S., Masafija– Jeknic, T., Sandine, W. E. & Giorannoni, S.J. (1995). An ecological study of lactic acid bacteria. *J.* Dairy Sci., 78: 1004–1017.

Schiffrin, E. J., Roulat, F., Link Amster, A., Aeschlimann J. M. & Donnet–Hughes, A. (1995). Immunomodulation of human blood cells following ingestion of lactic acid bacteria. J. Dairy Sci., 98: 491–97.