JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 63-68

Microbial Growth in Stored Human Breast Milk

O.F. Olukunle* and F.C. Adetuyi

Department of Microbiology, Federal University of Technology,
P.M.B 704, Akure, Ondo State, Nigeria.

(Received: 11 February 2008; accepted: 03 April 2008)

Investigation into the effect of storage period and temperature on the shelf life of Human Breast Milk (HBM) was carried out to assess the quality and fitness of expressed HBM after storage for 3 and 6 hours at 4°C and 26°C. The control sample was collected from a nursing mother using standard hygienic method while other samples were collected aseptically from nursing mothers, day care centers, crèches and various homes. The samples were conveyed to the laboratory for microbial analyses. There was no fungal growth observed in any of the samples. The mean bacterial count obtained for the fresh HBM was 1.53×10^3 and 1.85×10^3 Cfu/mL at 4°C and 26°C respectively after 3 hours of storage. At 4°C the bacterial load was 1.61×10^3 Cfu/mL and 2.16 x 103 at 26°C after 6 hours. Before and after 3 hours of the storage at 4°C, the control HBM had no microbial load, but has a value of 0.4×10^3 Cfu/mL at 26°C after 6 hours. The bacteria isolated from milk samples after storage are *Bacillus subtilis, Corynebacterium* spp., *Lactobacillus salivarus, Staphylococcus aureus, Staph. epidermidis, Lactobacillus acidophilus, Neisseria catarrhalis and Streptococcus lactis*. It is recommended that the length of storage should not exceed 6 hours at 26°C and 24 hours at 4°C.

Key words: Microbial Growth, Human Breast Milk.

Feeding is essential for growth and maintenance of body tissues. This is true in adults as well as infants. Balanced diets containing the essential ingredients will enhance proper growth and development in adults. Infants, however, are not able to feed on adult food because their digestive systems cannot effectively absorb them and it is very difficult to raise an infant on a non-milk diet in the first three months of his existence (Helsing and King, 1985). Among the different sources of milk, breast milk provides the best possible nutrition for both physical and mental development, supplying all the nutrients a baby needs for the first six months of life (Food and Drug Administration, 1998). Breast milk usually refers to the milk produced by a female animal which is fed to infants by breast feeding. The human breast milk (HBM) provides the primary source of nutrition for human newborns before they are able to eat and digest a wide variety of solid foods (May, 2007).

Vitamins are also preserved in breast milk but are destroyed by heating in formulas (TEF, 1998). Human breast milk contains a variety of immunological and antimicrobial factors that protect the body against infection and destroy harmful bacteria and viruses (May, 2005). Breast-fed babies are protected from a number of illnesses. Epidemiological studies have shown that breast feeding protects infants against respiratory and gastrointestinal infections, or decreases the severity of these infections. In addition, breast feeding protects babies against otitis media (middle ear infection), pneumonia, diarrhoea, necrotizing enterocolotis and sepsis (May, 2007). Studies have shown that the antimicrobial factors present in breast milk are not destroyed by pasteurization (62.5°C for 30 minutes). Mothers also produce antibodies to whatever diseases are present in their environment. Thus, human breast milk fights all

* To whom all correspondence should be addressed.

diseases inherent in their babies. A breast-fed baby's digestive tract contains a harmless bacterium called *Lactobacillus bifidus* that prevents the growth of more harmful organisms (TEF, 1998). When diarrhoea and other infections do occur, they are less severe among breast-fed infants and can be treated easily (Food and Drug Administration, 1998). Human breast milk is safe and clean. It never goes bad, even if the mother does not breast feed for some days (TEF, 1998).

Women who plan to go back to work soon after child birth would have to plan carefully if they want to breast-feed. The emancipation of women, however, had changed this accepted domestic role and encouraged women to seek salaried employment, usually distant from their homes. This change had long been established in industrialized countries, but now the urban areas in the developing countries are adopting it. As a result of current research, there had developed a reviewed awareness of the benefits of breast-feeding among women, even when working away from home (Williams, 1995). For example, almost all Nigerian mothers breast-feed their infants, although only about 2% of these infants who are younger than 2 months are exclusively breast-fed (human breast milk only). About 57% of them are fully breast-fed (with human breast milk and water), and the rest received other supplements apart from water (TEF, 1998). Accordingly, the Federal Ministry of Health and Social Services supported by UNICEF and WHO launched the Baby-Friendly Hospital Initiative Programme (BFHI) in order to protect, promote and support breast-feeding in the country (Onayade *et al*., 1996). To achieve the objectives of the programme, a number of teaching hospitals were designated as Baby Friendly Hospitals. The main objectives of the programme include promotion of early initiation of breast-feeding (within 30 minutes of delivery), exclusive breast-feeding for the first six (6) months of life; breast-feeding on demand, expression of breast-milk to feed infants while the mother is away; combination of breast-feeding with nutrient dense complementary foods well into the second year of life and beyond (Saadeh, 1993). The most viable option to make human breast milk available to infants while the mother is away is the expression and storage of human breast milk.

The nutritional content of breast milk makes it an ideal medium for the growth and proliferation of microorganisms. The organoleptic qualities of milk which include flavour, odour, microbial content, physical and chemical properties are changed usually due to presence of microorganisms in milk (Williamson, 1980). Bacteria of the genera *Lactobacillus* and *Streptococcus* are the natural microflora commonly associated with milk. They are referred to as lactic acid bacteria (Purohit, 1990). These bacteria are gram positive, non-motile, microaerophilic or anaerobic rods and cocci respectively. Other bacteria found in raw and unpasteurised milk are members of the genera *Pseudomonas, Staphylococcus* and *Bacillus*. Hawkers and Linton (1979), reported that milk drawn aseptically from the udder of cow shows a predominance of *Staphylococcus* species. However, yeasts are commonly found in raw milk or cream during hot weather which act upon the lactose to produce lactic acid and carbon dioxide (Purohit, 1990). Black, gray, green, blue or white moulds can be observed in milk (Helsing and King, 1985). They develop as fuzzy growth and discolour the milk and they are microbes used in cheese production. These include molds such as *Penicilium roqueforti* used in the production of Roquefort cheese. Milk is however, very difficult to maintain in the fresh state for long. Schroder (1982), noted that sources of contamination include milking equipment, handling personnel and the air in milking or its storage environment. Spoilage of milk is largely due to bacteria action. Perker and Collier (1990) revealed that *Streptococcus, Micrococcus*, *Lactobacillus* and *Bacillus* are found to be normal flora of man, animals, soil food, stuffs and other agricultural products. These may also find their ways into milk. Their presence as milk contaminants suggests that the milk samples are not processed under hygienic condition.

In the present study, we looked at different microorganisms associated with the keeping of human breast milk at low and room temperatures 4°C and 26°C which are the major temperatures at which HBM in Nigeria is commonly handled. This will give information to the type of milk spoilage and/or infections that babies fed with such milk can have. It will also

assist to enlighten and encourage women more about the importance of feeding babies with fresh milk.

MATERIAL AND METHODS

Nutrient agar (NA) and potato dextrose agar (PDA) were used for the isolation and identification of bacteria and fungal growth respectively.

Human Breast Milk (HBM) samples were collected aseptically from day care centers, crèches and various homes in Akure, Nigeria between 8.00 and to 9.00 a.m. using sterile pipettes. Samples drawn were transferred into sterile MacCartney bottles placed in iced bucket and transferred immediately to the laboratory within 30 minutes of collection. Control sample was collected from a nursing mother after the breast had been washed with soap and rinsed well. The areola and the nipple of the breast were cleaned with 70% ethanol. The milk was expressed with the use of pre-sterilized manual breast pump. Sample was collected into a pre-sterilized MacCartney bottle and then conveyed to the laboratory as other samples above. Each of the samples was divided into five portions. The first part was analyzed immediately, the second and third portions were stored at ambient temperature (26°C) while the fourth and fifth samples were stored at refrigeration temperature (4°C) for 3 hours and 6 hours respectively. They were diluted and pour plated using nutrient agar and potato dextrose agar which were incubated at 37°C and 27°C for 24 hours and 48 hours respectively.

RESULTS AND DISCUSSION

The effect of storage period and temperature on microbial load of the fresh samples is shown in Fig. 1. There was no fungus isolated from any sample either fresh or stored at refrigeration temperature (4°C) and ambient temperature (26°C) after 3 and 6 hr. There was no significant difference ($p<0.05$) in the bacterial population between fresh samples and those stored at 4°C for 3 hr while there was a considerable difference in the bacterial population of fresh and stored samples at 26°C ambient temperature for 3 hr. There was also no significant difference ($p<0.05$) in the bacterial load of all the samples except for samples 12, 13 and 14. The average bacterial count (1.53×10^3 Cfu/mL) for samples stored for 3 hr at 4°C was higher than that of the fresh samples (1.4×10^3 Cfu/mL). However, the average bacterial count (1.85×10^3 Cfu/mL) for samples stored at ambient temperature after 3 hr was the highest. After 6 hr of storage, the bacterial count of milk samples stored at 4°C increased to 1.61×10^3 Cfu/mL while that of the milk sample stored at ambient temperature increased to 2.16×10^3 Cfu/mL. There was no microbial growth in the control samples stored for 3 hr at 4°C and ambient temperature (26°C) but the least bacterial load was observed in it when stored at ambient temperature for 6 hr.

Low population of microbes in the control sample is in agreement with Helsing and King (1985) who reported that human breast milk collected straight from the breast is always sterile. The presence of microorganisms in other samples could therefore be as a result of the length of storage and the storage condition. This data is also similar to the findings of Barger and Bull (1987) who found out that human breast milk varies from mother to mother, and room temperature is often a subjective measure that varies over a period of time. The other reasons could be the nutrient content of human breast milk, its high water activity and its moderate pH that make the milk samples an excellent medium for the growth and proliferation of microorganisms (Adams and Moss, 2000). It was also observed that the bacterial load in each milk sample varied from sample to sample. This variation is because of the differences in the environmental factor, method of collection, containers used for storage, handling personnel, hygienic conditions of the mother at home and that of the nannies at the day care centers. The containers used for the expression of human breast milk samples were plastic and stainless cups with cover-lids. This is because most mothers find these containers more convenient and most day-care centers would not accept glass, which is considered the best choice because of the risk of breakage. Hammosh *et al*. (1996) reported that glass is usually considered the best choice for storing milk because the components of milk are

commensal of the respiratory tract, skin and conjunctiva. *Lactobacillus* species are found to grow freely in air. In air, there are many microorganisms especially in untidy and unhygienic environments. Air contains various types of microorganisms that are not its normal flora. These organisms found their way into the air through sneezing, coughing, talking and dust. This could be the reason why the types of organisms present in each sample vary between

Table 1. Morphological and biochemical characteristics of bacteria isolated from human breast milk

Isolates → Characteristics ↓	A	B	C	D	E	F	G	H
Gram stain	+	+	+	+	+	+	-	+
Shape of cells	R	LR	R	C	C	R	C	C
Spore stain	+	-	-	-	-	-	-	ND
Catalase	+	+	-	+	+	-	+	-
Motility	+	-	-	-	-	-	-	-
Coagulase	ND	-	-	+	-	ND	-	ND
Indole	-	-	-	-	-	-	-	ND
Oxidase	ND	-	-	-	-	-	+	ND
Methyl Red	ND	+	+	+	ND	ND	+	ND
Voges Prokauer	+	-	-	+	ND	ND	+	ND
Fermentation/Oxidation	ND	F/-	F/-	F/-	F/-	ND	-/O	ND
Fermentation of sugar:								
Lactose	-	-	+	+	+	+	-	+
Maltose	+	+	+	+	+	+	-	+
Sucrose	+	-	+	+	+	+	-	+
Arabinose	+	+	-	+	+	-	-	+
Fructose	+	-	+	+	+	-	-	ND
Galactose	ND	+	+	+	ND	+	-	-
Glucose	A	A	A	A	A	A	-	ND
Growth in 65% NaCl	ND	ND	ND	G	ND	ND	ND	ND
Temperature Tolerance 60%	+	ND	ND	ND	ND	ND	ND	ND
Mannitol salt agar	ND	ND	ND	FG	ND	ND	ND	G

A: *Bacillus subtilis*; B: *Corynebacterium* species; C: *Lactobacillus salivarus*; D: *Staphylococcus aureus*; E; *Stahylococcus epidermidis*; F: *Lactobacillus acidophilus*; G: *Neisseria catarrhalis*; H: *Streptococcus lactis*. +: Positive/present, -: Negative/absent, R: Rods, LR: Long rods, C: Cocci, ND: Not Determined, F/-: Fermentation only, -/O: Oxidation only, A: Acid Produced, FG: Fermentative growth, G: Growth.

Table 2. Frequency of occurrence of the bacteria isolated from human breast milk (HBM)

Bacterial species	Fresh Milk	Milk stored for:				Samples in which bacteria were present	Occurrence (%)
		3 Hours		6 Hours			
		4°C	26°C	4°C	26°C		
B. subtilis	+	-	+	-	+	1,14	3.9
Corynebact. spp.	+	+	+	+	+	5,8	13.2
L. salivarus	+	+	+	+	+	9,15	19.7
Staph. aureus	-	-	-	-	+	11,14	3.2
Staph. epidermidis	+	+	+	+	+	2,6,12	23.7
L. acidophilus	+	+	+	+	+	1,4,10,11	2.6
N. catarrhalis	+	-	-	-	+	2,6	23.7
Strep. lactis	+	+	+	+	+	3,7,13	2.6

+: Present, -: Absent.

individuals sampled in this work.

The keeping quality of fresh milk is partly a function of the temperature at which milk is kept and as such, low temperature storage is in wide application (Erwa, 1977). This work has revealed that storage temperature and the length of storage period affected the quality of the milk samples. The increase in microbial load over a storage period of 6 hr at ambient temperature is an indication for the presence of microbial spoilage (Adams and Moss, 2000). It is therefore important that storing milk at ambient temperature beyond 6 hr should not be encouraged for the infants. This is because consumption of microorganisms by these infants could lead to diarrhoea. This compares favorably with the information given by Barger and Bull (1987) on the length of time and storage temperature in which milk samples could be kept. It was stated that if milk samples is to be kept at 26°C, it should be consumed within 4 to 6 hr of storage or it could be kept at room temperature up to 8 hr (May, 2007). However, if it is to be refrigerated at 0-4°C, it should be consumed within 8 days. In all the samples kept at low temperature, the microbial counts obtained were still below the International Microbiological Standard, that is 10,000 Cfu/mL, which was recommended by FDA (1998).

ACKNOWLEDGEMENTS

We are thankful to the Department of Microbiology, Federal University of Technology, Akure, Nigeria for provision of materials and reagents used in this study. We appreciate the contributions of O. J. Olukunle and B. Boboye to the bench work and manuscript preparation of this article.

REFERENCES

1. Adams, M. R. and Moss, M. O., Food Microbiology, 2nd Edition Royal Society of Chemistry, Cambridge, U. K. 2000; 479.
2. Barger, J. and Bull, P. A., Comparison of the bacterial composition of breast milk stored at room temperature and stored in the refrigerator. *Int. J. Child Birth Education* 1987; **2**: 29-30.
3. Erwa, M. A., Modification of milk proteins by psychotropic bacteria. *J. Dairy Sci.* 1977; **60**: 857-861.
4. Food and Drug Administration, FDA Consumer Magazine, October 1998.
5. Hammosh, M., Lemon, J. and Todd, J., Breast-feeding and the working mother, effect of time and temperature on short-term storage of proteolysis, lipolysis and bacterial growth in milk. *Pediatrics* 1996; **97**(4): 492-498.
6. Hawker, E. L. and Linton, A. N., Microorganisms, 4th edn., Pergamons Press. 1979; 377: 619-630.
7. Helsing, M. and King, F. S., Breast-feeding in Practice. A Manual for Health Workers. Lower price edition, 1985; 211-217.
8. May, J. T., Breast Milk and Special Care Nurseries; Problems and Opportunities. Proceedings of 1995 Conference for Australian Bacterial Consultants Association (ACLA) Victoria Branch, Melbourne, 1997.
9. May, J. T., Retrieved from Science Technology and Engineering in January, 2005. "http: //en. Wikipedia.org/wiki/breast_milk", 2005.
10. May, J. T., Retrieved from Science Technology and Engineering in January, 2007. "http: //en. Wikipedia.org/wiki/breast_milk"; 2007.
11. Onayade, A. A., Davies-Adetugbo, Torimiro, S. A., Abejide, O. R., Adejuyigbe, E. A., Ononofua, F. T., Babajide, D. E., Obaniyi, J. O., Makanjuola, R. O., Breast-feeding; Knowledge, Attitude and Practices of Nursery Mother in Ife Central Local Government Area, Osun State, Nigeria; 1996.
12. Perker, M. T. and Collier L. H., Principle of Bacteriology, Virology and Immunity. Vol. II, 8th edn., 1990; 119, 161, 187.
13. Purohit. S. S., Microbiology. 4th edn., Mc Graw-Hill. 1990; 288-296.
14. Saadeh, R., Breast-feeding; The Technical Basis and Recommendation for Action General, WHO; 1993.
15. Schroder, M. J., Cousins C. M. and Mckinnon, C. H., Effects of psychotropic contamination on the keeping quality at 11°C and 50°C HTST of pasteurizing contaminated milk in the United Kingdom. *J. Dairy Res.* 1982; **49**: 610-630.
16. (TEF) Trinidad Express Feature. Tuesday, August 4, 1998; 1998.
17. William, M. R., Human Milk in the Modern New York. Oxford University Press; 1995.
18. Williamson, G. An Introduction to Animal Husbandry in the Tropics. 1980; 657-668.