Bacteriological monitoring of unheated human milk

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SUMMARY To assess the bacteriological quality of unpooled expressed breast milk, a pilot bottle sample of each donation was examined before the milk was given to the neonate. Provided the milk did not contain >2500 organisms/ml or potential pathogens it was used unheated. Milk containing between 2500 and 5000 organisms/ml and no potential pathogens was used after pasteurisation. Using these criteria, 67% of 460 donations were acceptable. However, because the bacteriological quality varied, 45% of domiciliary donations were discarded compared with only 29% of those from hospital.

Breast milk composition differs from cows' milk and artificial formulae in many important aspects. Some of these were described in the report from the Department of Health and Social Security (1974) which recommended that breast feeding should be encouraged. While human milk is generally accepted as ideal nutrition for term neonates, the composition varies and may not represent optimal nutrition for preterm infants and those of low birthweight. Davies (1977) showed that such infants fed artificial formulae had better rates of growth than those fed expressed breast milk. Nevertheless the importance of human milk to the young infant derives not only from its nutrient content but also from its 'anti-infective' properties. Lactoferrin, an iron-binding protein, is important in the prevention of infection by *Escherichia coli*; B₁₂ and folate-binding proteins which limit the availability of these vitamins for bacterial growth are also important. Specific IgA immunoglobulin acts in the neonatal gut and limits the growth and reproduction of bacterial and viral pathogens. Maternal antibodies, cellular content, bacteriostatic enzymes, lysozyme, and nonpathogenic bacteria further contribute to the anti-infective properties of human milk.

Carefully controlled pasteurisation reduces the damage to the humoral components of human milk, whereas higher temperatures not only destroy vegetative bacteria but can adversely affect the anti-infective properties and alter the chemical composition of the milk (Ford *et al.*, 1977; Williamson *et al.*, 1978).

In 1976 it was decided to examine the possibility of providing unheated, unpooled human expressed breast milk at this hospital for the nutrition of low birthweight babies, especially neonates in the intensive care unit, provided the bacteriological quality met our standards. Our experience of the bacteriological monitoring and provision of raw human milk is described.

Materials and methods

Only mothers who were free of communicable disease (for example, tuberculosis, respiratory infection, enteric, skin, or breast infection) and those not on drugs transmissible via breast milk were accepted as donors.

During a 2-year period (1976-78) samples of expressed breast milk (EBM) from hospital and from women at home were examined. All mothers received instruction on breast and hand hygiene and on the technique of manual expression of the breast, although most hospital specimens were obtained by the breast pump. After the EBM had been collected into autoclaved 100 ml glass bottles, approximately 2 ml milk was decanted into a sterile pilot bottle for bacteriological screening (Fig. 1). Those specimens collected in hospital were deep frozen to -20°C in the breast milk bank freezer as quickly as possible, often within a few minutes of collection. Specimens from home were stored in the ordinary compartment of the domestic refrigerator (not deep frozen) and taken to the breast milk bank in an insulated cool box every 48 hours by the donors themselves or by domiciliary midwives. On receipt at the hospital milk bank these domiciliary donations were then also stored at -20°C. Each bottle was stored separately and *not* pooled before freezing. Donations varied in volume between 50 and 100 ml

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Fig. 1 Expressed breast milk donation with accompanying pilot bottle sample.

and did not remain in the bank for more than a few days because of the demand for human milk. Before milk was issued, the pilot bottle was removed, allowed to thaw at room temperature, and then bacteriologically screened. This usually took less than 24 hours and no raw specimen of breast milk was used until bacteriologically acceptable. When clearance was given the donation was allowed to thaw at room temperature and issued for feeding. Thawed specimens were stored in the domestic compartment of the refrigerator in the intensive care unit until needed. Any thawed specimen not used after 12 hours (although rare) was discarded to reduce risk of bacterial contamination and denaturation.

The pilot bottle of milk was examined soon after receipt in the laboratory. Total quantitative bacterial counts on unheated samples were performed in duplicate using 10 drops from a standard 50-dropper pipette on a 1 in 5 dilution of milk in 1 in 4 strength Ringer's solution, whereas pasteurised milk was examined undiluted to increase the chance of isolating organisms which would be present in smaller numbers than in unpasteurised milk. In both instances, the milk was inoculated on to 10% horse

blood agar, MacConkey agar, 0.03% cetrimide agar, and nutrient agar containing 8.5% sodium chloride to facilitate the screening of organisms potentially detrimental to the safe use of milk. The sensitivity of the quantitative method meant that the minimum bacterial count detectable in unheated milk was 25 organisms/ml and in pasteurised milk it was 5 organisms/ml. Arbitrary criteria applied to the EBM samples were: (1) Milk with a quantitative count of <2500 organisms/ml (Wright, 1947) (consisting of, for example, micrococci, *Staphylococcus albus*, 'viridans type' streptococci, or diphtheroids which were considered to be contaminants probably derived from skin flora but unlikely to be pathogenic) was used unheated. (2) No donation was used unheated or pasteurised if the pilot bottle gave either a total count >5000 organisms/ml or any detectable potential pathogen. On an arbitrary basis the potential pathogens were defined as *Staphylococcus aureus*, β -haemolytic streptococci, *Pseudomonas* spp., *Proteus* spp., *Streptococcus faecalis*, and any other organism from a potential enteric or water-borne source (here defined as 'coliforms' for convenience). (3) No donated milk with a total bacterial count of 2500–5000 organisms/ml was used unheated. If the pilot sample had a bacterial count in this range, but none of the organisms listed in (2), the donated milk was pasteurised at 63°C for 30 minutes in a water bath and subjected to the same bacteriological screen, plus the alkaline phosphatase test. (Alkaline phosphatase is destroyed by a temperature of 63°C within 30 minutes and is used as evidence of adequate pasteurisation of cows' milk). Provided effective pasteurisation was established by no detectable growth on culture and a satisfactory phosphatase test, the milk was issued for use.

Results

The overall distribution of the bacterial counts of the EBM over the 2-year period is shown in Fig. 2. Clearly most EBM samples had low counts and the distribution shows gradually diminishing numbers of specimens up to 36 000 organisms/ml. Although there were 33 (4.5%) specimens with counts >50 000 organisms/ml, most (95.3%) specimens gave counts of <25 000 organisms/ml. A pilot investigation of anaerobic culture gave no additional information and was therefore stopped.

Of 688 raw EBM samples examined during the period, 513 (75%) came from hospital and 175 (25%) were domiciliary donations (Table 1). Within the three ranges of bacterial counts the figures for hospital and domiciliary isolates were similar—that is 460 (67%) of EBM samples gave counts of

Fig. 2 Distribution of bacterial counts in expressed breast milk over a 2-year period (1 May 1976–30 April 1978).

Table 1 Pattern of bacterial counts among hospital and domiciliary donations of expressed breast milk

Source	Bacterial count/ml			
	<2500	2500–5000	>5000	Total
Hospital	343 (67)	51 (10)	119 (23)	513
Domiciliary	117 (67)	15 (8.5)	43 (24.5)	175
Totals	460	66	162	688

Percentages are given in parentheses.

<2500 organisms/ml, and 66 (9.6%) specimens gave counts of 2500–5000 organisms/ml and were pasteurised if no pathogen was present. Overall 162 (24%) were immediately discarded without pasteurisation because of counts >5000 organisms/ml. Therefore, 526 (76%) EBM specimens had acceptable quantitative bacterial counts, but some of these had potential pathogens. The main pathogens isolated were *S. aureus* and 'coliforms', but there was a difference in the total number of isolates and in the type of organism from home and hospital donations. Altogether 49 (9.6%) of 513 donations from hospital were discarded, compared with 60 (34%) of 175 from homes. *S. aureus* was isolated from 23 (4.5%) of hospital donations and from 5 (3%) of home donations, whereas 'coliforms' were isolated from 21 (4.1%) of hospital and from 32 (18%) of domiciliary collections. There was no particular relationship between quantitative counts and potential pathogens, and overall the pathogens were distributed throughout the three quantitative ranges (Table 2).

Of the EBM donations with counts in the ranges of <2500 and 2500–5000 organisms/ml, 51 (7.4%) and 15 (2.2%) of them respectively had potential pathogens and were discarded without further

Table 2 Potential pathogens isolated from expressed breast milk samples

Organism and source	Bacterial count/ml			
	<2500	2500–5000	>5000	Total
<i>S. aureus</i>				
Hospital	15	5	3	23
Domiciliary	4	0	1	5
Coliform bacilli				
Hospital	8	2	11	21
Domiciliary	6	5	21	32
<i>Pseudomonas</i> spp.				
Hospital	0	0	1	1
Domiciliary	0	0	0	0
Faecal streptococci				
Hospital	0	0	0	0
Domiciliary	14	3	2	19
β -haemolytic streptococci				
Hospital	1	0	0	1
Domiciliary	3	0	1	4
<i>Proteus</i> spp.				
Hospital	0	0	3	3
Domiciliary	0	0	0	0
Totals	51	15	43	109

Table 3 228 discarded expressed breast milk donations because of unsatisfactory qualitative and quantitative results

Counts >5000/ml (n = 162)*		Potential pathogens (n = 66)*	
Hospital	Domiciliary	Hospital	Domiciliary
119	43	31	35

*Allowance has been made for 43 specimens discarded which had excessive counts and potential pathogens.

processing. Another 43 (6.3%) were discarded because they had excessive bacterial counts and potential pathogens.

The total number of EBM donations discarded because of excessive bacterial counts or the presence of potential pathogens was 228 (33%) (Table 3). The remaining 460 (66%) were acceptable because they contained no potential pathogen or excessive bacterial counts, as defined above, within the technical limitations of the methods employed.

Discussion

Like others, we experience difficulties in deciding what bacteriological criteria should be adopted to make EBM donations safe to give to neonates. We adopted arbitrary criteria based on such information as we could obtain. There appeared to be no widely agreed standards on the quantitative and qualitative content of human breast milk and no universally adopted figure for acceptable bacterial quality of EBM. There are no general guidelines on what are considered to be potential pathogens and the likely numbers of such species which are considered

harmful to neonates. There are opposing views on the benefits conferred on babies fed raw or pasteurised milk as well as conflicting results on babies fed artificial milk as opposed to EBM.

Nevertheless we decided to investigate the quantitative and qualitative bacteriology of EBM submitted to our maternity unit. It is not possible to give precise ranges of acceptable counts or to investigate which organisms constitute an unacceptable hazard to neonates, particularly those undergoing intensive care. The criteria we adopted, although arbitrary, were necessarily stringent in order to protect our neonates from any hazard of milk-borne bacterial disease. Even with these strict criteria we were still able to issue 67% of donated EBM, a figure similar to that of Williamson *et al.* (1978). Overall only 29% (150 of 513) of hospital donations were discarded compared with 45% (78 of 175) of domiciliary collections because their bacteriological quality was either quantitatively or qualitatively unacceptable. We feel that probably the collection procedure of the domiciliary EBM was the main cause for 45% of samples being unacceptable. If the donation was unsatisfactory the donor was again visited and given further advice about hygiene by the domiciliary midwife. To some extent this was done in the case of unsatisfactory hospital donations but an additional factor was the inadequate cleaning of the breast pump and related equipment. This defect was also noted by Liebhaver *et al.* (1978) who recommended manual expression of milk, thereby reducing the need for pasteurisation. Our figures also reflect the difference caused by lack of supervision of domiciliary collections, and if a neonatal unit relies heavily on domiciliary donations, closer supervision is necessary or it may be advisable to pasteurise all domiciliary donations. We should stress that our policy was devised to control the quality of the EBM and the whole procedure of collection, storage, etc. up to the time of feeding the neonate. The use of the pilot bottle provided us with a representative sample of EBM. We feel that if the donations had been pooled, even after being bacteriologically cleared, it would have added another factor to the procedure, with its attendant risks.

We did not attempt to assess the immunological or the nutritional benefits of using unpooled, unheated EBM, but we did try to assess clinically whether there was any hazard of infection. Clearly this is arbitrary, but we did not detect evidence of infection due to any of the organisms considered above in any baby fed EBM. We believe this was because EBM with potential pathogens was not used and also because EBM containing by definition nonpathogens <2500 organisms/ml does not expose the baby to any risk. In fairness it should be said that

if any baby is ill because of infection by an alleged nonpathogen (referred to earlier), it is extremely doubtful if the source of these ubiquitous organisms could be identified since they do not have a generally reliable marker system.

As a result of our experience in collecting and monitoring EBM, we have decided to continue to rely on raw EBM by fostering good hygiene technique which in most specimens yields low bacterial counts (Fig. 2). As a further safeguard, bacterial monitoring will exclude any donation of unsatisfactory quality. The development of this policy resulting from the ever pressing demand in our neonatal unit for EBM has led us to review our criteria in the light of these results and of other published work. We have considered the possibility of relaxing the quantitative criteria alone so that donations with higher bacterial counts (that is, those with >2500 organisms/ml) but no potential pathogens could be safely used. There is currently no generally agreed upper quantitative limit of likely nonpathogens, although most workers agree that it is inadvisable to use EBM containing potential pathogens. Ikonan and Maki (1977) used donations with counts <20 000 organisms/ml (some after heating) and discarded only 5% of all donations. If we had applied these criteria to our results in Fig. 2 we would have discarded only 10% of EBM donations on a quantitative basis alone. We are unwilling to accept EBM donations containing potential pathogens as we feel these could present unnecessary hazards to a vulnerable group of babies, particularly those undergoing intensive care. Milk containing bacteria which may be derived from a faecal origin is clearly unacceptable and these markers are used to exclude the possibility of contamination by enteric bacteria and viruses.

Breast feeding by a mother of her own normal baby will expose that baby to his own mother's bacterial flora which quantitatively may be >2500 organisms/ml, some of which may be potentially pathogenic. It is believed by some that these are probably less hazardous than the organisms from an unrelated milk donor, because a mother secretes antibodies in her milk which reflect her own immunological experience (Gunther, 1978).

As a result of the renewed interest in breast feeding and breast milk banks, attention has been given to the advantages and disadvantages of processing EBM. Because of the fear of contaminating EBM, attention has been directed to measures which provide a bacteriologically safe milk which still retains its immunological properties (*British Medical Journal*, 1977). However enterotoxins produced by certain bacteria—for example, *S. aureus* have caused outbreaks of food poisoning due to contaminated cows' milk (Wilson and Miles,

1975)—and since the enterotoxin is to some extent heat resistant it is theoretically possible that even pasteurised human milk, although free from bacteria, may contain enterotoxin.

In addition, because the alkaline phosphatase of human milk is 40 times lower than cows' milk (Heyndrickx, 1962) the phosphatase test as applied to the latter may not be sufficiently sensitive as an indicator of adequate pasteurisation of EBM. Therefore, although rigidly controlled conditions safeguard the pasteurisation process, bacteriological quality control criteria can provide a final check of effective pasteurisation.

Milk rendered safe by boiling or even by carefully controlled pasteurisation appears to reduce levels of immunoglobulin, lactoferrin, lysozyme, and complement C3 factor (Ford *et al.*, 1977; Evans *et al.*, 1978), as well as certain nutrients (Williamson *et al.*, 1978), although Raptopoulou-Gigi *et al.* (1977) in Edinburgh found that pasteurisation did not materially alter levels of IgA or lactoferrin. Total lymphocyte counts were also significantly decreased after pasteurisation (Liebhaber *et al.*, 1977). These findings indicate the reduction of anti-infective properties of EBM with pasteurisation, and by avoiding this procedure we feel that unheated EBM of acceptable bacteriological standard provides a practical alternative for a baby in place of its own mother's milk. However, in spite of the advantages of the immunological content of nonpasteurised EBM we agree with Lucas *et al.* (1978) that it cannot be recommended for all special care baby units unless there are certain quality control safeguards.

Milk macrophages tend to adhere to the walls of the glass container and therefore simple storage may reduce the lymphocyte content. Moreover, Pitt (1976) suggested that freezing of milk will markedly diminish the number of viable cells and it must be admitted that either of these factors could have reduced the effective immunological value to our babies. Quantitative examination of the noncellular components of milk deep-frozen for 3 months at -20°C had no appreciable effect on levels of lactoferrin, lysozyme, IgG, IgA, or C3 complement component (Evans *et al.*, 1978). Repeated freezing and thawing would be expected to lead to reduction of these levels because of denaturation, but in our milk bank the process only happens once.

While cellular transfer in the breast milk from the natural mother may confer immunological advantages to her baby, it may be potentially harmful in an unrelated baby. Beer and Billingham (1975) reported a wasting syndrome similar to a graft v. host disease when neonatal rats were fed on the milk of an unrelated rat. Further work is needed

to find out if this observation is important to human studies on unheated EBM.

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