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Collection methods and contamination of bank milk

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SUMMARY Bank milk collected by manual expression was less likely to be contaminated than milk collected by other methods. Contamination with coliforms and gentamicin-resistant Gram-negative rods was related to the pumps used by donors in their homes. Stringent precautions reduced but did not eliminate contamination.

Bacterial contamination of human milk is a well-known cause of neonatal illness. To evaluate the risk of contamination associated with commonly used collection methods, we related the bacterial content of frozen bank milk to the method of collection.

Methods

Donors were highly motivated unpaid volunteers. Donors were asked to wash their breasts and nipples as tolerated as well as their hands before each collection. Initially, plastic breast cups (Netsy cups) were used during breast feeding to collect drips from the contralateral nipple. The cups were washed before breast feeding and the milk transferred to sterile bottles immediately after collection. All other milk was collected by manual expression, Egnell electric pump, or Loyd B hand pump (Figure);

ordinary hand pumps were not used.¹ Collection technique was rediscussed with any mother who submitted contaminated milk.

All milk was collected in the donors' homes, transported weekly from the donors' home freezer (about -20°C) to the milk bank, pooled using sterile technique, and refrozen at -20°C . A sample from each pool was diluted 10^2 , 10^4 , and 10^6 in Mueller Hinton broth and plated on to 5% sheep's blood agar and eosin-methylene blue agar. Aerobic colony counts were made after 24 and 48 hours' incubation at 36°C on blood agar and eosin-methylene blue agar. Gram-negative isolates were further screened by triple sugar iron, urea, Simmons citrate, lysine iron agars, and motility-indole-ornithine medium. Organisms other than *Pseudomonas aeruginosa* showing no activity on triple sugar iron were identified only as 'non-fermenters'. Gram-positive isolates were screened on blood agar and coagulase testing, mannitol salt agar, or optochin discs as needed. Quantitative bacterial counts were made on blood agar and methylene-blue plates. After gentamicin-resistant Gram-negative rods (GNR) had been identified in several pools, all milk was cultured on brain heart infusion plates containing $8\text{ }\mu\text{g/ml}$ gentamicin. Any pool containing *Staphylococcus aureus*, coliforms,

Figure Lloyd B hand pump (upper) and Egnell electric pump (lower).

pneumococci, group B streptococci, gentamicin-resistant GNR, or a total of $>10^5$ colonies/ml was considered contaminated.

If no source of contamination could be identified in our bank, electric pumps were cultured. Donors had been previously instructed to boil the external parts for 10 minutes each day. The trap bottle was boiled only if overflow occurred. After the cultures were performed, donors were told to boil all external parts including the trap bottle after each use, and to rinse the tubing with vinegar.

Lloyd B pumps were distributed to some donors after contamination was linked to electric pumps. Donors were told to boil the breast cup and collection bottle 10 minutes after each use. Our data were analysed by χ^2 analyses. The time before gentamicin-sensitivity was routinely evaluated, donor instructions modified, and before contaminated pumps

were gas autoclaved was designated as period 1. Period 2 referred to the subsequent period.

Results

As shown in the last line of the Table Netsy cups were associated with the highest percentage of contaminated pools ($P<0.01$). The overall rate of contamination associated with Egnell pumps was reduced during period 2 ($P<0.05$). An intermediate percentage of pools collected by Lloyd B pumps was contaminated. Different species of gentamicin-resistant non-fermentative GNR were found in pump-collected pools.

Manually expressed pools were less often contaminated than pools collected by the Egnell pump during period 1 ($P<0.05$). Gentamicin-resistant GNR were not identified in manually expressed milk, unlike milk collected by Lloyd B pump or by Egnell pump during period 1 ($P<0.01$). This difference occurred even though the number of donors and volume per pool of manually expressed milk (6.1 donors; 105 oz) exceeded that for the Lloyd B pump (5.1 donors; 89 oz) and the Egnell pump (2.2 donors; 69 oz).

All 7 electric pumps contained GNR. Gentamicin-resistant GNR were identified in 4 pumps and coliforms in 1. The nipples of 5 donors were cultured. Two donors had gentamicin-sensitive GNR. None

Table Bacterial content of milk pools

Collection method	Netsy cup Period 1	Egnell pump		Lloyd B pump Period 2	Manual expression, both periods
		Period 1	Period 2		
Pools	5	37	42	31	61
Median total colony count ($\times 10^3$ /ml)	82	5.5	0.8	1.8	1.3
Median Gram-negative colony count ($\times 10^3$ /ml)	75	1.1	<0.1	0.4	<0.1
Percentage of pools containing					
<i>Staphylococcus aureus</i>	60	0	5	0	5
Coliforms	20	24	10	16	7
$\geq 10^5$ colonies/ml	40	14	5	3	7
<i>Staphylococcus aureus</i> , coliforms, or $\geq 10^5$ colonies/ml	80	30	17	19	13
Gentamicin-resistant GNR	—	29*	2	10	0†
Gentamicin-resistant GNR <i>Staphylococcus aureus</i> , coliforms, or $\geq 10^5$ colonies/ml	—	50*	17	23	10†

*Stains in 14 pools tested for gentamicin sensitivity. † Stains in 39 pools tested for gentamicin sensitivity.

had gentamicin-resistant GNR. No GNR were identified in oropharangeal cultures of the 5 donors' infants. The pumps were used to collect fresh milk from the 5 donors. Gentamicin-resistant GNR ($>10^7$ colonies/ml) were identified in the milk collected by 1 mother. Gentamicin-sensitive GNR were identified in the milk ($>10^5$ colonies/ml) but not on the nipples of another donor.

The median colony count in 2 previous studies of milk collected by Egnell pump^{2,3} exceeded our values, even during period 1. *Klebsiella* sepsis has been linked to contaminated breast milk feeds collected by Egnell pump.⁴ Human milk feeds have also been incriminated in other neonatal infections.⁵⁻⁷

Discussion

Breast pumps, especially Egnell pumps, are widely used, and few centres culture milk collected by mothers for their infants.

Strict precautions will reduce but not eliminate the risk of contamination during collection of human milk.

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