



## Prevalence of bacterial contamination of powdered infant feeds in a hospital environment

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**Background.** The study arose as part of a best-practice nutrition model regarding the introduction of ready-to-use (RTU) infant feeds in place of powdered infant feeds (PIFs) as a standard formula for infants under the age of 1 year who are unable to be breastfed. Internationally and locally there is grave concern regarding the safety and efficacy of mixing PIFs, especially in a hospital setting, and the resultant bacterial contamination causing enteric infections, especially in premature, immunocompromised and sick infants.

**Objective.** To evaluate the prevalence of bacterial contamination of PIFs given to infants at Red Cross War Memorial Children's Hospital, Cape Town.

**Methods.** Quantitative levels of bacterial contamination were determined and were expressed as colony-forming units (CFUs) per millilitre of sample. Aliquots of milk were inoculated onto agar, and the milk samples were then incubated at 25°C overnight ( $N = 10$ ), 30°C overnight ( $N = 48$ ) and 30°C for 6 hours ( $N = 34$ ). Post-incubation milk samples were cultured again.

Contamination was defined as any positive culture before administration (i.e. pre incubation) or  $> 10^2$  CFU/ml after administration (i.e. post incubation).

**Results.** Fifty samples of PIFs ( $N = 82$ ) were contaminated pre incubation, with 25/82 samples (30.4%) being heavily contaminated ( $\geq 10^4$  CFU/ml). Post incubation, 43/92 samples (46.7%) were contaminated with  $> 10^2$  CFU/ml. The acidified PIFs appeared to have some bactericidal effect against some of the organisms, but not all.

**Conclusions.** RTU infant feeds are sterile and are recommended for use in all hospitalised infants. The results of this study indicate that even when milk is prepared in a controlled environment there is significant bacterial contamination of PIFs post production. As RTU feeds are now readily available in South Africa every attempt should be made to use a sterile RTU system for hospitalised infants.

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The World Health Organization (WHO) recently expressed concern regarding the safe handling, preparation and delivery of powdered infant feeds (PIFs) in health care settings. PIFs are not sterile and may contain pathogens such as *Salmonella*, *Enterobacter sakazakii* and other enterobacteriaceae. PIFs have been associated with serious illness and death in some infants.<sup>1-5</sup>

It is postulated that only small numbers of micro-organisms are required to cause illness. This risk escalates if milk is held at ambient temperatures for long periods, particularly in a hot climate, allowing bacteria to multiply.<sup>1,2</sup> This practice is common in a local context where warm, freshly prepared

PIFs may be left at high ambient temperatures to cool before refrigeration.

A significant association has been found between the extent of bacterial contamination and the presence of diarrhoea. The administration of contaminated feeds to patients may result in the patients being colonised by opportunistic pathogens or endotoxins. In one study<sup>6</sup> enterobacteriaceae were found in one-third of all positive cultures. Septicaemias, enteral sepsis, diarrhoea and abdominal distension have all been described.<sup>7,8</sup>

Previous recommendations from Codex Alimentarius allowed for minimal contaminants in PIFs. More recent recommendations from this body<sup>9</sup> indicate that no level of pathogenic organisms in a PIF can be considered safe or acceptable. However, current manufacturing techniques do not allow for the production of sterile powders and as such PIFs may always contain some pathogenic organisms.<sup>1,2</sup>

A local study, which considered a sub-cohort of the mothers receiving infant milk from the National PMTCT Cohort, found that 70% of the mothers within a community were using feeds that were too old to be safe, 64% of the milk was contaminated with *Escherichia coli*, and in 26% of the milk *E. coli* was found to be at a concentration  $> 10^4$  CFU/ml (unpublished data).

New recommendations for the production of PIFs in a hospital environment include reconstituting the powder using water at 70°C, which should then be cooled using a blast

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refrigerator chiller to 4°C within ½ an hour of preparation. PIF should be transported to wards using a refrigerated trolley at 4°C and stored immediately in a dedicated industrial fridge capable of maintaining a temperature of 4°C or less, for a maximum of 24 hours. It should be consumed within 1 hour if the infant is drinking directly from the bottle, and within 4 hours if being enterally fed. PIFs should not be warmed before administration as a continuous enteral feed.<sup>1,2</sup>

In addition, strict traceability is recommended. Each bottle should be labelled with the name of the formula, the infant's name and hospital number, and the time and date of preparation and the preparer's name. If this cannot be achieved in a hospital setting then use of sterile ready-to-use (RTU) feeds should be considered in all infants, especially those considered to be at high risk for infection.<sup>1,2,9</sup>

RTU formulas may be stored at room temperature and do not require refrigeration unless opened; because of their sterility they are regarded as the safest way in which to provide non-contaminated nutrients to patients.<sup>1,2,9</sup>

## Objective

As part of a best-practice model the aim of this study was to determine the prevalence of bacterial contamination of the then current standard feed at Red Cross Hospital, e.g. an acidified PIF (Pelargon, Nestlé). While there is a significant body of international data<sup>1-5</sup> on the microbial contamination of PIFs there is a paucity of local data to support the use of RTU infant feeds over the current PIF alternative.

## Methods

### Specimen collection

Five millilitre aliquots of reconstituted PIFs were taken using aseptic technique by two of the authors (LM and EG) on three different occasions. The same observers sampled the milk on all three occasions, using sterile syringes to aspirate 5 ml aliquots from the bottles before distribution to the wards. The bottles to be sampled were chosen at random on all three occasions, thus representing formula to be used by a variety of patients in the hospital. The milk kitchen staff were unaware of the study in process. In total, 82 PIF samples were analysed, with 34 samples collected on the first occasion, 10 on the second and 38 on the third.

The samples were transported to the microbiology laboratory at ambient temperature, and arrived in the laboratory no more than 1 hour after collection. On arrival, samples were processed immediately.

### Laboratory procedures

On arrival in the laboratory, 100 µl aliquots of the milk were inoculated onto 2% sheep blood agar and McConkey agar.

The agar plates were incubated at 37°C overnight. In order to simulate conditions in the wards, the milk samples were held at various conditions. The first 34 samples were held at 30°C for 6 hours. Samples in the second batch of 10 were divided, and held at both 25°C and 30°C overnight. The final batch of 38 was held at 30°C overnight. In total, therefore, 34 samples were held at 30°C for 6 hours, 48 samples were held at 30°C overnight, and 10 samples were held at 25°C overnight.

After being held at the respective temperatures and times, 100 µl of the sample was inoculated onto media as described above. There were a total of 82 samples inoculated pre incubation, and 92 samples inoculated post incubation.

The agar plates were incubated at 37°C aerobically for 24 hours, at which stage the plates were examined and the number of colonies counted manually. If no growth was visible after 24 hours the plates were incubated for another 24 hours and re-examined. Organisms were identified to genus or species level by Gram's stain appearance and standard biochemical tests. The number of organisms were expressed as colony-forming units (CFUs) per millilitre of sample. Antimicrobial susceptibility testing was not performed on the organisms isolated.

## Ethics

Approval for the study was obtained from the University of Cape Town Research Ethics Committee.

## Results

A significant level of contamination was defined as any positive microbial culture before incubation (i.e. on arrival in the laboratory) or as contamination with 10<sup>2</sup> CFU/ml after incubation (i.e. after simulated ward conditions). These cut-off criteria relate to bacterial contamination of grade A pasteurised milk in a healthy population<sup>8</sup> and do not relate to premature or sick infants. Codex Alimentarius<sup>9</sup> recommends zero pathogen contamination as being the acceptable measure.

Three sets of samples were collected (on three different occasions), and each set was held at different simulated ward conditions. The results were pooled, and are presented in Table I. In summary, 50 of the 82 PIF samples (60.9%) that were cultured before incubation showed some degree of contamination, with 20/82 samples (24.4%) being heavily contaminated ( $\geq 10^4$  CFU/ml). The range of organism cultured included *Bacillus* spp. ( $N = 12$ ), *Acinetobacter* spp. ( $N = 31$ ), mixed growth ( $N = 6$ , *Acinetobacter* and *Bacillus*) and coagulase-negative staphylococcus ( $N = 1$ ). After incubation, 74/92 samples (80.4%) were contaminated; 43/92 of the samples (46.7%) showed significant growth  $> 10^2$  CFU/ml. Twenty-five of the 92 samples (27.1%) had growth  $> 10^4$  CFU/ml. The acidified PIFs appeared to have some bactericidal effect against some of the organisms, but not all.



Table I. Pooled results of culturing PIF samples before and after simulated ward conditions

Growth (CFU/ml)	Pre-incubation (N = 82)		30°C, 6 hours (N = 34)		30°C, 12 hours (N = 48)		25°C, 12 hours (N = 10)		Post incubation (all samples, N = 92)	
	%	(N)	%	(N)	%	(N)	%	(N)	%	(N)
No growth	39	(32)	47	(16)	4	(2)	0	(0)	20	(18)
0 - 10 <sup>2</sup>	31	(25)	47	(16)	21	(10)	50	(5)	33	(31)
10 <sup>2</sup> - 10 <sup>4</sup>	6	(5)	6	(2)	33	(16)	0	(0)	20	(18)
> 10 <sup>4</sup>	24	(20)	0	(0)	42	(20)	50	(5)	27	(25)

## Discussion

An open system uses either RTU milk decanted from a can/tin, and/or powdered feeds that are reconstituted using water or occasionally milk.<sup>7</sup> A closed system relates to where a product is spiked with an administration set and hung in the manufacturer's container. Numerous studies have shown that open systems present a contamination risk for sick patients in a hospital setting who are already compromised.<sup>6</sup>

Enteral feeds are a fertile media for bacterial growth and have been identified as a significant source of nosocomial infection. Work has shown that 25 - 62.5% of patients receiving formulas prepared at central sterile services department or ward level respectively were colonised by organisms initially isolated from cultures of the formulas.<sup>11</sup>

The current study found that 30% of the PIFs sampled showed heavy bacterial contamination even in newly prepared feeds. The degree of contamination increased if the milk was left to stand for a length of time in a warm environment, as would be expected. Even if left for only 6 hours, the number of samples showing bacterial contamination increased from 8/34 to 18/34. If samples were left longer, the degree of bacterial contamination increased further, for example 25/48 samples were contaminated (> 10<sup>2</sup> CFU/ml) before incubation at 30°C for 12 hours, and following incubation this increased to 36/48.

Of concern was the significant contamination of PIFs on production, with 20 samples containing > 10<sup>4</sup> CFU/ml. For an average 3 kg infant receiving 55 ml x 8 infant formula per day, this equates to the oral ingestion of 4.5 million pathogenic organisms per day, which is likely to result in severe gastroenteritis or other nosocomial infections.

A significant association has been found between the extent of bacterial contamination and the presence of diarrhoea.<sup>11</sup> In one study<sup>12</sup> enterobacteriaceae were present in one-third of all positive cultures. Septicaemias, enteral sepsis and diarrhoea in addition to abdominal distension have all been described.

Hospital practices often contribute to PIF becoming contaminated. Such practices include use of manipulated feeds, the addition of modular additives and adding additions or mixing formulas at ward level. Even where there are written procedures they are not always understood or followed.<sup>13,14</sup>

On average feeds are kept at room temperature for approximately 2½ hours before refrigeration. This time period is associated with suitable conditions for microbial growth. In a previous study<sup>11</sup> colony counts of 10<sup>2</sup> - 10<sup>3</sup> CFU/ml were found just after preparation, increasing to 10<sup>8</sup> - 10<sup>10</sup> CFU/ml after exposure to room temperatures. Our study was not designed to detect such high colony counts as it was felt that any growth > 10<sup>4</sup> CFU/ml would be potentially significant.

It is recommended that procedures that spare nursing time and enable safe practice should be adopted. The use of closed-system sterile formula feeding containers have been shown to reduce both contamination and nursing and preparation time. It takes a nurse an average of 2 minutes per patient per day to manage a closed enteral feeding system, compared with 14 minutes per patient per day for an open system.<sup>8,13,14</sup>

In terms of nutrition content PIFs confer the same benefits as RTU infant feed equivalents. However, the advantages of a closed system outweigh any that can be documented for an open system. When all cost implications are considered, e.g. labour, time, resources and medical costs, it is more cost effective to provide a RTU system. The use of RTU feeds results in reduced costs from fewer ventilator days, fewer complications, fewer resources and reduced time of skilled personnel required.<sup>14</sup>

We have estimated that with the implementation of the closed infant feeding system at Red Cross Children's Hospital we are able to render a saving of R1 643 536 per annum compared with managing an open PIF system, taking into account the salaries of nursing staff required to run the milk kitchen, general assistants required to wash all the bottles, nursing time to hang the feeds based on data arising from other studies,<sup>12,13</sup> and dietetic time required to calculate 'special' feeds. This costing did not take into account any decrease in length of hospital stay, fewer antibiotics and/or ventilator days.

## Conclusion

Sterile RTU infant feeds provide a microbial-safe cost-effective alternative to PIFs and should be recommended for use in all tiers of the district health system. Their use results in cost savings on length of hospital stay, antibiotic use, morbidity and mortality. RTU infant feeds do not require chilling, a dedicated



industrial fridge, or refrigerated transport. Their use decreases nursing time, results in less wastage, improved quality assurance and control of infant feed contents and delivery of a superior cost-effective nutrition service.<sup>11-15</sup>

This study forms part of a best-practice model, supported by the management of Red Cross War Memorial Children's Hospital. As a result of this support we have managed to decommission the milk kitchen, switching over to RTU infant feeds for all infants in the organisation. While PIFs play an important role in a community setting in the case of infants whose mothers are unable to breastfeed, we do not feel that they currently have a role to play in a setting where there are sick hospitalised infants.

The results from this study indicate that even when milk is prepared in a controlled environment there is significant bacterial contamination of PIF post production. As RTU infant feeds are now readily available in South Africa it may not be judicious to continue to use PIFs for sick and premature infants in a hospital setting, and every attempt should be made to switch to a sterile RTU system.

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