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Microbial evaluation of infant milk formula

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In developing countries like Pakistan use of Powered Infant Formula (PIF) is widespread in order to provide nutrition for infant, one of the more vulnerable groups in our society. Contamination of PIF during manufacturing and use is a point of concern. Recently there has been considerable concern related to the presence of bacteria in PIF, in particular *Enterobacter sakazakii, Salmonella, Bacillus cereus*, *Staphylococcus aureus.* The present project has been designed to evaluate microbial contamination of six selected brands of infant formula marketed in Pakistan. Microbial analysis confirms that among 6 samples 2 were contaminated with *Bacillus subtilis and* 4 samples were not contaminated. However, all of them are within the range of Codex Alimentations Commission. During the present study all samples were not found to be contaminated with *Enterobacter sakazakii, Salmonella* that are potential powdered infant formula-borne pathogens.

Keywords: Powered Infant Formula, microbial contamination, infant formula-borne pathogens

INTRODUCTION

The quality of infant feeding is of paramount importance for growth, development, and long term health well into adulthood (Secretariat, World Health Organization 24 Nov 2001). Breastfeeding is recognized as the ideal form of infant feeding, providing multiple benefits for child health (Hoffman J,. 2003-08-07).Thus breast feeding should be actively promoted, protected, and supported. Infants who cannot be fed at the breast or for whom breast milk is not available need infant formula milks of high quality (Prentice A,. December 1996). An artificial substitute for human breast milk.

The Codex Alimentations Commission, part of both the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization, develops standards, guidelines, and related texts on food to protect consumers' health and to ensure fair trade practices globally. Its standard on infant formula was adopted in 1981, based on scientific knowledge of the 1970s, and it is currently being revised. Infant formula must be the sole source of nutrients for several months during a critical phase of growth and development, and thus it must meet very high quality standards (U.S. FDA Infant Formula).

Use of infant formula has been decreasing in industrial countries for over forty years as a result of antenatal education, increased understanding of the risks of infant formula, and social activism. A 2001 report of the WHO strongly advocates breastfeeding over the use of infant formula except in unusual circumstances (Secretariat, World Health Organization 24 Nov 2001)

Reasons Of Formula Feeding:

Due to the mother's health: The mother is infected with HIV or tuberculosis. She is malnourished or has had certain kinds of breast surgery. She is taking any kind of drug that could harm the baby (Centers for Disease Control and

Prevention (2006-08-26). The baby is unable to breastfeed: The child has a birth defect or inborn error of metabolism such as galactosemia that makes breastfeeding difficult or impossible.

Due to absence of the mother: The child is adopted, orphaned, or in the sole custody of a man. The mother is separated from her child by being in prison, or a mental hospital the mother has abandoned the child.

IV. Due to family pressures: Family members, such as husband, encourage use of infant formula.

V. Due to under education: The mother believes that her breast milk is of low quality or in low supply, or that breast feeding will decrease her energy, health, or attractiveness. The mother is not trained sufficiently to breastfeed without pain and to produce enough milk (Centers for Disease Control and Prevention (2006-08-26).

Due to financial pressures: Maternity leave is unpaid, insufficient, or lacking. The mother's employment interferes with breastfeeding.

VII. Due to societal structure: Breastfeeding is difficult or forbidden at the mother's job, school, place of worship or while commuting. The mother feels infant formula is socially prefer able.

VIII. Due to dietary concerns: The contents of breast milk are influenced by the dietary habits of the mother. If the mother consumes a food that contains an allergen breastfeeding may, for a brief period after consumption, provoke an allergic reaction in the infant (Centers for Disease Control and Prevention (2006-08-26).

IX. Due to lifestyle: In some cultures (e.g., nomadic North African tribes) infants do not receive sufficient sunlight to generate Vitamin D (to protect the baby from the elements the mothers always keep the child wrapped in cloth). Infant Formula can be fortified with Vitamin D (Centers for Disease Control and Prevention (2006-08-26).

Types Of Infant Formula:

There are three major classes of infant formula: (Fomon, Samuel J,. 2001).

Milk-based formulas

Prepared from cow milk with added vegetable oils, vitamins, minerals, and iron. These formulas are suitable for most healthy full-term infants.

Soy-based formulas

Made from soy protein with added vegetable oils (for fat calories) and corn syrup and/or sucrose (for carbohydrate). These formulas are suitable for infants who cannot tolerate the lactose in most milk-based formulas or who are allergic to the whole protein in cow milk and milk-based formulas.

Special formulas

for low birth weight (LBW) infants, low sodium formulas for infants that need to restrict salt intake, and "predigested" protein formulas for infants who cannot tolerate or are allergic to the whole proteins (casein and whey) in cow milk and milk-based formulas.

Forms Of Infant Formula:

Infant formula is available in three forms: Ready-to-feed, Concentrated Liquid and Powder. Ready-to-feed is used "as is." Concentrated liquid (the only liquid that comes in a 13 ounce can) and powder must be diluted with water according to instructions on the label. Ready-to-feed and concentrated liquid infant formulas are commercially sterile. Powdered formulas are not sterile. Preparation of any form of infant formula (especially powdered products) requires careful handling to prevent contamination and minimize growth of microorganisms.

Microbial aspects of powered infant formula

Powered infant formula has been safely consumed by infants for more than 50 years and constitutes over 80% of the infant formula used worldwide. The powered form offers some advantages compared to the liquid form particularly for its lower cost. However, it is important to note that powered infant formula meeting current standards is not a sterile product and may contain low levels of (opportunistic) pathogens. It is not feasible, using current processing technology, to eliminate completely the potential for microbial contamination. Available scientific information reveals that, contamination of powered infant formula (PIF) with Enterobacteriaceae (or coliforms) and Salmonella has been a cause of colonization, illness and death in infants.

The group at particular risk is infant (i.e. children<1 year).Among infants, those who are immune compromised and neonates (<28 days) are considered to be at greatest risk, particularly neonates of low birth weight (<2500g).infants of HIV-positive mothers are also of concern because they may specifically require infant formula and may be more susceptible to infections (Enterobacteriaceae and other microorganisms in powder infant formula: meeting report, MRA

series 6, ISBN: 9241562625 (WHO).

The microorganisms or microbial toxins of concern with powdered infant formula, and the strength of the evidence of a causal association between their presence in powdered infant formula and illness in infants, were categorized as follows:

Category "A" Organisms – Clear Evidence Of Causality

Enterobacter sakazakii and *Salmonella enterica* are in category "A" because both are well-established causes of illness in infants (e.g. systemic infection, necrotizing enterocolitis [NEC] and severe diarrhea), and they have been found in powdered infant formula. Contaminated powdered infant formula has been convincingly shown, both epidemiologically and microbiologically, to be the vehicle and source of infection in infants.

The presence of *E. sakazakii* in powdered infant formula (and its association with illness in Infants) is more likely than other Enterobacteriaceae or other *Enterobacter* species to be detected, because of the paucity of other vehicles or modes of transmission for *E. sakazakii* in this age group, and because it is facilitated by the use of molecular fingerprinting detection techniques. In other words, there may in fact be more instances of powdered infant formula-borne infection with Enterobacteriaceae than with *E. sakazakii,* but the former elude detection. Although there are clearly some differences in the microbial ecology of *S. enterica and E. sakazakii,* many of the risk-reduction strategies aimed at controlling *E. sakazakii* are also likely to control other Enterobateriaceae, especially other Enterobacter species.

Category "B" Organisms – Causality Plausible, But Not Yet Demonstrated

Other Enterobacteriaceae are in category "B" because they are well-established causes of illness in infants (e.g. systemic infection, NEC and severe diarrhea) and have been found in powdered infant formula, but contaminated powdered infant formula has not been convincingly shown, either epidemiologically or microbiologically, to be the vehicle and source of infection in infants. These organisms include, for example: *Pantoea agglomerans* and *Escherichia vulneris (both formally known as Enterobacter agglomerans), Hafnia alvei, Klebsiella pneumoniae, Citrobacter koseri, C. freundii*, *Klebsiella oxytoca and Enterobacter cloacae.* These organisms are increasing in importance as neonatal pathogens and, being Enterobacteriaceae (known to be present in low levels in powdered infant formula), are potential candidates as powdered infant formula-borne pathogens. For example, infant formula has been implicated as the vehicle of infection in an outbreak of *C. freundii* infection (Thurm and Gericke, 1994). In this event, however, it was not shown how the feed became contaminated.

Category "C" Organisms – Causality Less Plausible Or Not Yet Demonstrated

Other microorganisms are in category "C", either because, despite causing illness in infants (e.g. systemic infection, NEC and severe diarrhea), they have not been identified in powdered infant formula, or, although having been identified in powdered infant formula, they have not been implicated as causing such illness in infants. These organisms include *Bacillus cereus, Clostridium difficile, C. perfringens, C. botulinum, Staphylococcus aureus* and *Listeria monocytogenes. Bacillus cereus*, a spore-forming gram-positive rod commonly found in the environment, is an acknowledged enteropathogen. Enterotoxigenic B. cereus has been isolated from reconstituted milk-based formula (Rowan and Anderson, 1998). Although one confirmed common source outbreak associated with infant formula has been reported in Chile (Cohen et al., 1984), no evidence of intrinsic contamination of the infant formula with B. cereus was provided. Thus, a causal association between powdered infant formula and B. cereus infection was not demonstrated. *Clostridium difficile* is a frequent colonizer of newborns, usually without clinical manifestations. One study, sparked by the finding of stools positive *for C. difficile* in two infants dying of sudden infant death syndrome (SIDS), showed significantly greater colonization of newborns fed on formula than breastfed infants (Cooperstock et al., 1982). However, no direct link with powdered infant formula was established.

Contamination during manufacturing:

Dry infant formula is manufactured according to three process types:

Wet-mix process:

All ingredients are handled in a liquid phase and heat-treated (critical control point [CCP]), e.g. pasteurized or sterilized, and then dried.

Dry-mix process:
Individual ingredients are prepared, heat-treated as appropriate, dried and then dry-blended.

Combined process:

Part of the ingredients is processed according to (a), in order to produce a base powder to which the rest of the ingredients are added according to (b).

The main microbiological issues of current public health concern associated with powdered infant formula are related to the presence of *Salmonella* and other Enterobacteriaceae (coliforms) including *E. sakazakii*. The presence of these microorganisms may occur as a result of:

Contamination through ingredients not submitted to a heat treatment during the powdered infant formula manufacturing process (this applies for dry-mix and combined processes).

Contamination from the processing environment during the dry steps of the process, i.e. contamination post-thermal processing, presumably acquired from the processing environment during drying or packing (this applies for dry, wet and combined processes).

Contamination during reconstitution and use:

Reconstituted PIF is nutritious and can support bacterial growth when given favorable conditions of water availability, time and temperature. Therefore, once dehydrated the only remaining barriers to increased bacterial growth and risk of infection, are time and temperature.

Temperature Of Reconstitution

When the temperature of mixing water is 10 or 20^{o}C, minimal growth of microorganisms will occur. However, subsequent holding for long periods at room temperatures can result in growth and therefore increase risk.

When the temperature of mixing water is 30 or 40^{o}C no inactivation of *E. sakazakii* will occur, however conditions are favorable for growth. Therefore, in cases where the formula is not consumed immediately, quick cooling to lower temperatures is required to minimize growth. Re-warming followed by holding or holding at room temperature for an extended period (even in the absence of re-warming) may result in growth and therefore increase the risk.

When the temperature of the mixing water is 50^{o}C, no appreciable inactivation occurs and therefore risk is determined by the amount of growth that can occur. As a result quick cooling to lower temperatures is required to minimize growth.

In the case of the 60^{o}C mixing water temperature, some initial inactivation occurs but depending on the particular preparation scenario this inactivation can be overwhelmed by the magnitude of growth that may occur if temperatures permit, for example during extended feeding times.

With reconstitution using 70^{o}C mixing water, significant inactivation occurs.

Holding Time

Increased holding times at room temperature result in large increases in risk, as a result of the growth that occurs. This effect is exaggerated for warmer room temperatures.

The same holding times at refrigeration temperatures indicated less than 1.3 fold risk increase.

Personnel

The fecal carriage of the organism has not been demonstrated and so it is uncertain how often contamination of PIF may occur during preparation. However, it is well accepted that b asic aspects of personnel hygiene are frequently ignored and poor hygienic practice has been the probable source of outbreaks (Block C., Peleg O,. et al., 2002, Clark N.C,. et al., 1990).

Salmonella & enterobacter sakazakii

Salmonella and *Enterobacter sakazakii* are the microorganisms of greatest concern in infant formula. Contamination of powdered infant formula with *E. sakazakii* and with salmonellae has been the cause of infection in infants, sometimes with serious sequelae or death. *Salmonella and E. sakazakii* do not survive the pasteurization processes used during manufacture but recontamination of the powdered infant formula during handling and filling processes may occur. *E. sakazakii*, due to its ubiquitous character, seems to be more difficult to control in the processing environment than *Salmonella*. Environmental microbiological testing in the processing area is necessary to monitor the effectiveness of the hygiene measures. Testing the processing environment for Enterobacteriaceae is the most effective method of monitoring the efficacy of processing and hygiene since Enterobacteriaceae are more often present than *Salmonella* and *E. sakazakii. Salmonella* and *E. sakazaki*i can grow in the reconstituted product if stored above 5 ^{o}C for a

sufficient time and multiply very rapidly at room temperatures. Good Hygienic Practices at reconstitution, storage and feeding are essential to avoid recontamination and/or multiplication of the pathogens in the reconstituted formula. The most effective control measure to minimise risks of *Salmonella and E. sakazakii* in high-risk infants (pre-term, underweight, immunocompromised), would be to use commercial sterile liquid formula.

It is recommended that a Performance Objective (PO) for powdered infant formula and follow-on formula, aiming at very low levels of *Salmonella and E. sakazakii* (e.g. absence in 1, 10 or 100 kg) is introduced and that verification of compliance with the PO is confirmed by testing for *Enterobacteriaceae* in the environment and in the product. In addition it is recommended that guidelines for preparation, handling, storage and use of infant formula in the home and in hospitals are developed.

Microbiological specification

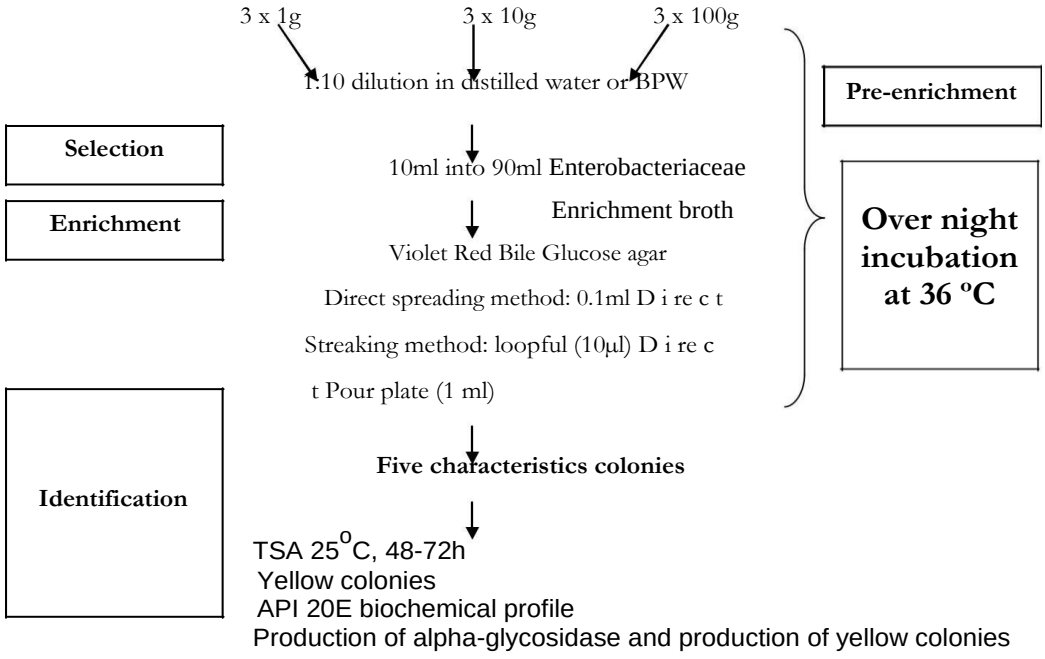
Microbiological specifications should be established in the context of risk management options. A number of factors will have an impact on the level of microorganisms found in reconstituted powdered infant formula. Steps should be taken during manufacturing to minimize the likelihood that microorganisms of concern (e.g., *Salmonella and E. sakazakii*) will be present. The current Codex microbiological specifications relating to mesophilic aerobic bacteria, coliforms and *Salmonella* for powdered infant formula (CAC/RCP 21-1979).These criteria are to be applied to the finished product (powder form):

Experimental work:

For the microbiological evaluation of infant formula imported and locally manufactured brands of six formulas were procured from the market and evaluate according to current Codex of microbial specification of powered infant formula (PIF) (CAC/RCP 21-1979).

Preparation of sample:

The procedure of evaluation by USFDA was used. Samples were prepared and inoculated. Direct spreading, streaking and direct pour method were used. The flow diagram of procedure is given below.



** Each formula was tested 3 times
** For Salmonella 25 g of each sample was used.

Ref: Quantitative E. sakazakii isolation procedure. a USFDA (2002); b Muytjens, Roelofs-Willemse, and asper (1988); c Nazarowec-White and Farber (1997b) from Iversen and Forsythe (2003).

RESULTS AND DISCUSSION:

During the procedure selected six samples of powered infant formula were procured from the market for microbial evaluation both locally and imported manufactured as given in microbial specification USFDA that not less than six samples are to be used.

Each sample was given a code No. in order to maintain the secrecy and results of evaluation are given in Table No. 1 - 4.

TABLE NO. 1 BACTERIOLOGICAL GROWTH ON TSA

S NO	CODE NO	NO OF COLONIES	TYPE OF ORGANISIM
1	A	NIL	------
2	B	NIL	------
3	C	10	Gram +ve rods
4	D	NIL	------
5	E	NIL	------
6	F	8	Gram +ve rods

TABLE NO. 2

BACTERIOLOGICAL GROWTH ON MacConkey AGAR

S NO	CODE NO	NO OF COLONIES	TYPE OF ORGANISIM
1	A	NIL	------
2	B	NIL	------
3	C	NIL	------
4	D	NIL	------
5	E	NIL	------
6	F	NIL	------

TABLE NO. 3

BACTERIOLOGICAL GROWTH ON EEB

S NO	CODE NO	NO OF COLONIES	TYPE OF ORGANISIM
1	A	NIL	------
2	B	NIL	------
3	C	NIL	------
4	D	NIL	------
5	E	NIL	------
6	F	NIL	------

TABLE NO. 4 COMPOUND RESULTS

S NO	CODE NO	E-coli	Staphylococcus	Enterobacter sakazakii	Salmonella	Bacillus
1	A	NIL	NIL	NIL	NIL	------
2	B	NIL	NIL	NIL	NIL	------
3	C	NIL	NIL	NIL	NIL	+ve
4	D	NIL	NIL	NIL	NIL	------
5	E	NIL	NIL	NIL	NIL	------
6	F	NIL	NIL	NIL	NIL	+ve

The purpose of present study, evaluating microbial contamination of Powered Infant Formula (PIF) marketing in Pakistan is to first establish whether contamination were present and what sort and numbers, secondly to determine which organisms are present and whether they represent a potential hazard either contaminated during manufacturing or reconstitution point of view.

Babies deserve the best of everything that can be offered. The first year of life is the most critical for a baby, particularly from a nutritional standpoint. During the initial and most crucial months of growth and development in a child's life, nutrition and diet should be a major concern to parents. Choosing an infant feeding method, whether breast milk or baby formula, is ultimately the parent's decision.

A century ago, babies who couldn't be breast-fed usually didn't survive. Today, although it is recognized internationally that breast milk is the best source of nutrition for infants. However, there are instances where it may be insufficient or not available and thus, may need to be supplemented or replaced. In those instances, one of the dietary options is the use of powdered infant formulae (PIF).However, Infant formula is a close enough second that babies not only survive but thrive.

It is not possible using current technology to produce powdered formulae that are devoid of

Low levels of microorganisms, i.e., the products cannot be sterilized. Thus, their microbiological safety requires strict adherence to good hygienic practices during both manufacture and use.

Powdered infant formula is manufactured by more than a dozen firms in 40 – 50 processing plants worldwide. Although there are many plants that produce this product, the manufacturing processes are very similar. Powdered infant formula is manufactured using two general types of processes: a dry blending process and a wet mixing – spray drying process. Some manufacturers use a combination of these processes. In the combined process, a base powder (consisting mainly of protein and fat components) is produced using the wet mixing and spray drying process and then the base powder is dry blended with the carbohydrate, mineral and vitamin ingredients. These processes have different risks and benefits with respect to the potential for product contamination by bacteria such as *Enterobacter sakazakii* or other harmful bacteria.

However, Manufacturers of powdered infant formula are upgrading their technology and committed to providing a safe, high quality product and have responded to the challenge of eliminating formula borne pathogens by implementing more stringent process controls. These include the use of extremely rigorous hygiene practices, labels with explicit instructions for product preparation, and specific measures to satisfy HACCP. Additionally, most manufacturers have developed more stringent microbiological standards than those of the FDA and the FAO/WHO Codex Alimentarius, namely no coliform cells in 25 grams (0 cfu/25g) of powdered formula. While implementing this standard has probably helped reduce outbreaks, evidence shows that it has not completely eliminated them. Despite of them, bottle feeding has some advantages like,

Anyone can feed the baby with the bottle at any time. This may also give your partner chance to be more involved in the feeding process.

Formula usually takes longer to digest than breast milk, so the time gap between feeding is longer and babies need to eat less often.

The mother can go about her daily chores, or to work without frequent feeding intervals or pumping. She can also leave the child under the care of her partner, or other caretaker. In public, bottle-feeding is much more convenient.

With bottle-feeding, you know how much milk your baby is drinking.

With breastfeeding the mother has to take precautions regarding her diet, fluid intake, medications, clothes etc. With baby formula, those restrictions do not exist and she can resume normal life much sooner.

To meet the objective of present study, six samples of powdered infant formula were selected for microbial evaluation (Table 1-4).2 samples out of 6 were contaminated with gram +ve rods but are within the range of the current Codex microbiological specifications *for* powdered infant formula (CAC/RCP 21-1979).

Moreover, all six samples were not contaminated with gram –ve strains not detected by EEB and MacConkey media recognized selective media to detect gram-ve pathogens.

The present results reveal that the newborn infant is so susceptible to infections that PIF requires a high level of microbiological quality control during production, distribution and usage.

CONCLUSION

The conclusion of the present study all samples

were free from contaminate like *Enterobacter sakazakii, Salmonella* that are potential powdered infant formula-borne pathogens. Our samples safe from infectious contaminate. It is important to ensure that PIF is prepared using good hygienic procedures, along minimization of the time between preparation and consumption to reduce the risk of contamination

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mr. Imran designed and performed the experiments and also wrote the manuscript, Dr. Samiyah Tasleem reviewed the manuscript and Prof. Dr.Syed Baqir S.Naqvi supervisor this study. All authors read and approved the final version.

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