Microbes in Pediatric Infant Formula

Adeel Arsalan [1,*], Zubair Anwar [1], Iqbal Ahmad [1], Arif Saba [1], Syed Baqar Shayum Naqvi [2]

[1] Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Toll Plaza, Super Highway, Gadap Road, Karachi-74600, Pakistan.
[2] Department of Pharmaceutics, Faculty of Pharmacy, University Of Karachi, Karachi-75270, Pakistan.

ABSTRACT

Pediatric infant formula (PIF) is of immense importance for the cognitive and psychomotor development of infants and young childrens. Although mother-fed is one of the precious gifts for infants from nature. Hence, world renowned regulatory bodies like World Health Organization (WHO), Health Canada, Food and Drug Administration (FDA) of USA, Medicine and Health care Product Regulatory agency (MHRA) of UK strongly recommends breast-feed due to the possibilities of microbial contamination in infant formula.

Although PIF is frequently used a number of microbes like Enterobacter sakazakii, Salmonella enterica, Staphylococcus aureus, Bacillus cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Citrobacter freundii, and Klebsiella pneumoniae are found in PIF. Among these Enterobacter sakazakii, Salmonella and Clostridium species, Citrobacter freundii, and Bacillus cereus are highly virulent and may cause several lifethreating illnesses to neonates and infants like necrotizing enterocolitis, systemic infections, severe diarrheas, and allergies. It is difficult to prepared sterile powdered infant formula. Therefore, the quality of PIF should meet very high quality standard. Moreover, some probiotics like Bifidobacterium and Lactobaccili species are usually added for the beneficiary effect. These probiotics aid in the digestion, stimulate the immune system, and inhibit the growth of pathogens, effective against bacterial induced gastroenteritis, and even recovery from acute diarrhea in children mainly associated by Escherichia coli, Salmonella and Shigella species.

Key Words

Pathogenic bacteria; Contamination; Hazards; Probiotics.

Correspondence to:

Abdel Aleem A. El- Saba
Department of Cytology and Histology;
Faculty of Veterinary Medicine;
Cairo University.
E-mail: adeelarsalan@hotmail.com

1 Introduction

The quality of infant feeding is of paramount importance for growth, development, and long term health well into adulthood (WHO, 2001). It is not possible by using current technology to produce pediatric infant formula (PIF) that is devoid of low levels of microorganisms. Ready-to-feed liquid infant formula may not contain contamination due to sterilization whereas PIF is not sterile. PIF must be the sole source of nutrients for several months during a critical phase of growth and development, and thus it should meet very high quality standards. Ready-to-feed and concentrated liquid infant formulas are commercially sterile while PIF are not sterile. A number of microbes such as Bacteroides, Bifidobacterium, Clostridia, Lactobacilli, and Streptococci are been found in PIF (Stark PL and Lee A., 1982; Benno Y, Sawada K, Mitsuoka T., 1984; Harmsen HJM. et al., 2000). The gut of the fetus is sterile at time of birth. The baby acquires a complex collection of microorganisms within hours which colonize in the mouth and then finally the whole gut. The multiplication of definite microorganisms is influenced by certain factors such as environmental contact, the infant's diet, mode of delivery and microbiota of motherfeed (Fanaro S, Chierici R, Guerrini P, Vigi V, 2003). In normal vaginal delivery, an infant is exposed to the mother's vaginal

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and fecal flora, which may results in the colonization of Escherichia coli and Lactobacilli and Bifidobacterium species. While in case with caesarian section a variety of microbes are acquired from surgical tools. Therefore, in both the cases the sterile digestive tract has been contaminated (Holzapfel WH. et al., 1998; Mountzouris K, McCartney A, Gibson G, 2002; Collins MD, Gibson GR, 1999. Rotimi and Duerden observed that first Lactobacilli and Enterobacteria formed colonies in the gut due to abundant oxygen. When the oxygen is mainly consumed by these bacteria, obligate anaerobes are multiplied (Rotimi VO, Duerden BI, 1981).

Various studies have been supported motherfed over PIF (Goldman AS, Chheda S, Garofalo R, 1997; Cuthbertson WJF, 1999; Garofalo RP, Goldman AS, 1999). WHO strongly recommends mother-fed for atleast six months to infants (WHO/UNICEF, 2003). The pH of the stomach of the infant is initially is less acidic but due to presence of Streptococcus and Lactobacillus, and their metabolic activities create a more acidic environment (Berseth CL, 2006). Mother-feed rates differ from one region to other region. Motherfeeding rate is 95% and less than 30% in European and Scandinavian countries respectively. There is high rate of motherfeeding of 6 months old child in Scandinavian region as compared to European countries. Barash et al. have been found that approximate 77% samples of PIF are contaminated by Clostridium species including presumptive Clostridium perfringens (Barash JR, Hsia JK, Arnon SS, 2010).

In 2002, Mountzouris with his co-workers found the major difference between the microbial flora of breast-feed and top-feed. Breast-fed infants digestive tract is mainly colonized by Bifidobacterium, but the human milk contains antibacterial factors which may help in the less prone to infections due to a large amount of Bifidobacterium. Moreover, the antimicrobial factors also inhibit the growth of facultative anaerobes while in the case with formula-fed the gut of infant is predominantly colonized by Bacteroides with some Bifidobacterium. Due to the lack of natural antimicrobial agents in PIF, infants are more prone to infections due to the lower amount of Bifidobacterium. This may result in a higher risk of diarrhea and allergies (Mountzouris K, McCartney A, Gibson G, 2002).

Reasons of the Use of Pediatric Infant Formula

The main reasons for using PIF are as follows. Mother may be infected by herpes simplex, chickenpox, HIV and tuberculosis or drink alcohol at high level and malnourished or breast surgery (FAO/WHO, 2004; Lawrence RM, Lawrence RA, 2004). Wahl with his colleagues in 2012 recommended that HIV infected mother could fed their babies because the virus are killed by the components present in breast milk (Wahl A. et al., 2012). It has been observed that due to risk of malnutrition like iron deficiency, vitamin deficiency and inadequate nutrition by foods (Spitzer AB et al., 2001; Mamiro PS. et al., 2005). Some regulatory bodies like Health Canada strongly suggest the addition of vitamin D in PIF, even breast-fed infants must receive supplemental vitamin D. Some families believe that bottlefed may rise father's role in parenting the infant (Earle S., 2000). Various others studies supported that neurological benefits of breast milk remain, regardless of dioxin exposure (Rogan WJ. Et al., 1991; Brouwer A. et al., 1998).

1.1 Hazzarderous Microbes in Pediatric Infant Formula

The manufacture of commercially sterile PIF is not practicable by using current processing technology; there are potential risks of infection to infants through consumption of PIF. These risks are increased when PIF is prepared, handled, and/or stored not appropriately. The microbes and its toxins are of major concern in PIF; their presence may cause illness and even death of infants. It has been found that the addition of sugar product in PIF may increase the risk of contamination of product.

These hazards of the invasion of the organisms were categorized as A, B, and C. Enterobacter sakazakii and Salmonella enterica are in category "A" in which clear evidence of causality, because both are major causes of sickness in infants (e.g. necrotizing enterocolitis, systemic infection, and severe diarrhoea) (Lai KK., 2001). Microbial contaminated powdered infant formula has been persuasively revealed, both epidemiologically and microbiologically, to be the vehicle and source of infection in infants. There may in fact be more occurrences of PIF borne infection with Enterobacteriaceae than with E. sakazakii. There are clearly some differences in the microbial ecology of Salmonella enterica and E. sakazakii; many of the risk-reduction strategies aimed at controlling E. sakazakii are also probable to manage other Enterobateriaceae, especially other Enterobacter species.

Pantoea agglomerans, Escherichia vulneris, Klebsiella oxytoca, Hafnia alvei, Citrobacter koseri, Citrobacter freundii, Klebsiella pneumoniae, and Enterobacter cloacae are categorized as "B" which may cause causality plausible, but not yet demonstrated. PIF has been concerned as the vehicle of infection in an outbreak of Citrobacter freundii infection (Thurm V and Gericke B., 1994).

The microorganisms causing causality less plausible or not yet demonstrated are categorized as "C" organisms. These organisms include Staphylococcus aureus, Bacillus cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, and Listeria monocytogenes. Bacillus cereus, a spore-

forming gram-positive rod commonly found in the environment. B. cereus has been isolated from reconstituted milk-based formula which may produce Enterotoxigenic (Rowan NJ, Anderson JG., 1998). Clostridium difficile is a frequent colonizer of infants and may cause pseudomonas colitis (Limaye AP. et al., 2000).

Storage and Handling of Pediatric Infant Formula

Farmer et al. reported 57 strains of E. sakazakii has been shown growth at 25˚C, 36˚C and 45˚C out of 57 only 50 strains strains have grown at 47˚C, but none of strain has shown multiplication at 4˚C or 50˚C (Farmer JJ. et al., 1980). It has been found that minimum growth temperatures for E. sakazakii in Brain Heart Infusion broth varied from 5.5˚ to 8˚C and at 4˚C microbes has been started to kill while show major growth at temperature 41˚C to 45˚C (Nazarowec-White M., Farber JM, 1997). Iversen with his co-workers have observed that improper storage of contaminated reconstituted powdered infant formula might help rapid growth of E. sakazakii. The incubation time for E. sakazakii in reconstituted PIF were 13.7 hours, 1.7 hours and 19-21 minutes at 6˚C, 21˚C and 37˚C, respectively (Iversen C, Lane M, Forsythe SJ., 2004).

Commonly Isolated Bacteria in Pediatric Infant Formula

Bifidobacterium Species

Gram-positive anaerobe Bifidobacterium mainly colonize in the infant's intestine rather than stomach because of less oxygen supply. Bifidobacterium species are found if the infant is on either breast-fed or formula-fed. The most common Bifidobacterium species found in infant's intestine are Bifidobacterium infantis, Bifidobacterium breve, and Bifidobacterium longum. Bifidobacterium infantis is specifically unique to the infant's digestive tract (Matsuki T, Watanake K., Tanaka R., 2003). Bifidobacterium helps in the digestion of glucose and oligosaccharides, which not only provide energy and nutrients for growth but also help in eradication of Clostridium species (Ward RE., et al., 2006). It has been observed that by addition of probiotics in PIF reduces the pH of of infant's stool like the pH of breast-fed infants, indicates the growth of beneficial microbes like Bifidobacterium (Costalos C., 2007).

Lactobacilli Species

Lactobacilli are Gram-positive rods commonly found in the whole gut but mainly present in the large intestine and mainly used as probiotics (Tannock GW., 2004; Wall R., et al, 2008). Lactobacilli are capable to stay alive and show growth even at pH 3.7 to 4.3 in fermented milks and yogurts. Lactobacilli are more acid tolerant as compared to Bifidobacterium. Lactobacilli stimulate the immune system, help in digestion, and inhibit the growth of pathogenic bacteria such as Helicobacter pylori by decrease in pH of stomach due to accrual of lactic acid (Haarman M., Knol J., 2006. Parracho et al. have observed that Lactobacilli also hamper the growth of other bacteria by contending with them for nutrients and hold the place on the epithelial lining of the intestine (Parracho H, McCartney A, Gibson G., 2007). Gonzalez with his colleagues has found that mixture of both Lactobacillus species are used as bacteriotherapy against the three diarrheacausing microbes (Isolauri E. et al., 1991) It has been proven that the nearly all probiotics reduced diarrhea and gastroenteritis in infants (Parracho H, McCartney A, Gibson G., 2007; Isolauri E. et al., 1991; Saavedra JM. Et al., 1994; Isolauri E. et al., 1995; Engelbrektson A. et al., 2009). It has been found Lactobacillus acidophilus is added in PIF to improve weight gain of infant (Isolauri E. et al., 1991; 1995). Infants are mainly suffered from watery diarrhea and/or excessive flatulence. Lactobacillus species have been increased Beta-galactosidase (lactase) which may develop lactose digestibility (Rastall RA. et al., 2000).

Enterobacter Sakazakii

Enterobacter sakazakii is a Gram-negative, non-spore forming Enterobacteriaceae (Farmer JJ, 1980). Pitout et al. have been reported the resistances of E. sakazakii to many antibiotics like penicillin and its derivatives (Pitout JD. Et al., 1997). A number of studies have been found the contamination of E. sakazakii in PIF (Biering G. et al., 1989; Simmons BP. et al., 1989; Van Acker J. et al., 2001). The less acidic environment of stomach of premature babies is an important factor for the survival of Enterobacter sakazakii (Van Acker J. et al., 2001; Muytjens HL. et al., 1998). Noriega and co-workers have observed formula preparation equipment might be contaminated by E. sakazakii and supported by Block et al. (Noriega FR. Et al., 1990; Block C. et al., 2002). Enterobacter sakazakii is ubiquitous and chiefly found in food processing area, milk powder production area as well as in households utensils (FAO/WHO, 2004; Iversen C., Forsythe S., 2004; Kandhai MC. Et al., 2004). Muytjens and Kolle have found no isolation of Enterobacter sakazakii in environment, including soil, surface water, mud, grain, bird droppings, rotting wood, domestic animal's milk (Muytjens HL., Kollee LA., 1990) but can be found from the hospital environment (Masaki H., 2001). E. sakazakii has also been isolated from clinical sources like blood, sputum, CSF, intestinal and respiratory tracts, inflamed appendix tissue, bone marrow, urine, eye, ear, wounds, and stool (Adamson DH., Rodgers JR., 1998; Gallagher PG., Ball WS., 1991; Gurtler JB. et al., 2005). E. sakazakii infections have not only been occurred in infants but may also occur in adults (Lai KK., 2001). Immuno-compromised infants

and neonates are considered to be at greatest risk, especially neonates of low birth weight and pre-mauture (Block C. et al., 2002; Centers for Disease Control and Prevention (CDC), 2002; Bar-Oz B. et al., 2001). Pagotto with his colleagues have been first illustrated the virulence factors for E. sakazakii. Some strains of E. sakazakii have been produced enterotoxin compounds which may produce a cytotoxic effect (Pagotto FJ., 2003). HIVpositive mother's infants are also of great concern, because may particularly need PIF and more at risk of infection. Infants may suffer the rate of E. sakazakii infection was 0.001% while the rate among low-birth-weight neonates was 0.0087% (WHO, 2004). 50% to 80% of PIF is the main direct or indirect source of E. sakazakii induced infections. Enterobacter sakazakii is chiefly associated in life-threatening meningitis, cerebritis, necrotizing enterocolitis and septicemia in infants (Lai KK., 2001). Enterobacter sakazakii are highly virulent pathogens survive in macrophages because some strains of E. sakazakii may form capsules (Pagotto FJ., et al., 2003). E. sakazakii can affix to intestine and even plastic and silicon surfaces and multiply in a biofilm. Feeding bottles and nipple can offer biofilm for the increase in E. sakazakii count (Zogaj X., 2003).

Bacillus cereus

Becker with his colleagues has been reported that about 70% PIF are contaminated by Bacillus cereus in 1992, but in 1994, only 18% of PIF was contaminated with Bacillus cereus. It has been proved that the processing and packaging practices in the PIF manufacturing plant have been improved to reduce microbial contamination. It has been found that heat treatment initiates the production and germination of Bacillus cereus spores. During pasteurization, elevated temperature for short period of time may provide the milk as good germination medium, even if the PIF is placed in desiccator, the spores may also survive (Becker H. et al., 1994). Stadhouders and his co-workers have investigated the major sources of contamination of B. cereus spores, due to biofilm did not remain clean on the surface of stainless steel processing equipments; spores are formed in milk before pasteurization and may stay alive through the heating process and preserve in the dried milk (Stadhouders J, Hup G., Hassing F., 1982).

Other Pathogens

In various studies, it has been found Salmonella contamination in PIF (Usera MA. Et al., 1996., Threlfall EJ. et al., 1998; Olsen SJ. et al., 2001; Bornemann R. et al., 2002). Rates of salmonellosis are the most commonly observed in infants as compared to any other age group (Olsen SJ. et al., 2001). Umoh et al. have reported that S. aureus survive without reducing the count in PIF from day of opening to approximate 12 days (Umoh VJ, Obawede KS., Umoh JU., 1985). Staphylococci multiply within three hours and produce enterotoxins in heat treated milk (Gosh SA., Laxminarayana H., 1973). Muytjens et al. have been reported the presence of 52% Enterobacteriaceae but found not any Salmonella species in 141 different PIF from 35 countries. There is intrinsic or extrinsic contamination of Citrobacter freundii in PIF (Muytjens HL., et al., 1988).

Health Risks Associated to Pediatric Infant Formula

A number of studies have been reported that there is a huge risk of the use of PIF. The chances life threatening illnesses like gastroenteritis, respiratory tract infections, acute otitis media, diabetes, necrotizing enterocolitis, obesity, eczema, asthma, atopic dermatitis, and even infant death syndrome may be associated with contaminated PIF Stanley IP., et al., 2007; Riordan JM., 1997; Sadauskaite-Kuehne V. et al., 2004). McCann and Ames have

Table 1. Probiotics in Pediatric Infant Formula.

Probiotics	Effect of Probiotics
Bifidobacterium animalis	Effect on the gastrointestinal system
Lactobacillus acidophilus	Reduce the side effects of antibiotic therapy (Engelbrektson A. et al., 2009)
Lactobacillus johnsonii	Reduce inflammation and the incidence of Helicobacter Pylori (Sgouras DN. et al, 2005)
Lactobacillus reuteri	Used in H. Pylori infection (Saggioro A, et al., 2005) Beginning confirmation for diarrhea improvement in children (Ruiz-Palacios G, Guerrero ML., Hilty M., 1996).
Listeria innocua or Listeria monocytogenes	Reduce symptoms of lactose intolerance and immune stimulation (Sellars RL., 2007).
Mixture of Lactobacillus acidophilus and Lactobacillus casei	May affect digestive system (Millette M, Luquet, FM., Lacroix M., 2007).
Mixture of Lactobacillus acidophilus and Bifidobacterium bifidum	Evidence for reduced Clostridium difficile associated disease (Mcfarland LV., 2006).
Lactobacillus rhamnosus GG (LGG)	Help in acute diarrhea in children (Isolauri E. et al., 1991) Reduce episodes of relapsing diarrhea caused by Clostridium difficile toxin (Hilton E. et al., 1997) Reduction in the extent and intensity of atopic dermatitis (Kalliomaki M. et al., 2003).
Supplementation with Lactobacillus rhamnosus GG and with Bifidobacterium bifidum and Streptococcus thermophilus	Preventing rotavirus diarrhea in infants (Saavedra JM. et al., 1994).
Lactobacillus casei	Recovery from acute diarrhea in children mainly caused Escherichia coli, Salmonella, and Shigella species (Isolauri E. et al., 1995).

found by the iron supplementation in PIF the chances of delay neurological development and may decrease I.Q (McCann JC., Ames BN., 2005). Stanley et al. have not found any relation between iron and neurodevelopment. E. sakazakii and S. enterica, and C. botulinum spores are found in honey added to PIF leading to infant intestinal botulism (Stanley IP., et al., 2007). Townsend with his co-workers has found lipopolysaccharide is a heat stable endotoxin that persists during the processing of PIF. There is huge risk of neonatal bacteraemia and endotoxemia, especially in neonates with immature immune systems (Townsend S. et al., 2007)

Heat Treatment

Some studies have been showed that standard pasteurization practices are effective for the inactivation of E. sakazakii (Iversen C, Lane M, Forsythe SJ., 2004; Nazarowec-White M, McKellar RC., Piyasena P., 1999). The ability to be osmotolerant may increase the risk of the organism becoming more dominant in the environment, thus increasing the risk of postprocessing contamination of powdered infant formula. Kandhai et al. have been found that after pasteurization equipment used in the manufacturing of PIF may be contaminated, if the equipment is not well cleaned and maintained (Gurtler JB, Kornacki JL., Beuchat LR., 2005).

Microbial Aspects of Manufacture and Use of Pediatric Infant Formula

There are many ways by which PIF can be manufactured, so a number of possibilities in the contamination of microbes. PIFs are mostly manufactured by dry-mix method, wet-mix method, and combination of both methods.

The main reason of the contamination in PIF is mainly through ingredients which are not exposed to heat and contaminated through the processing environment during drying and packing (FAO/WHO, 2007).

Some Commonly Used Microbes in Pediatric Infant Formula

Some bacteria which are non-pathogenic, nontoxic and exert a beneficial effect on the host are commonly used, as probiotics in PIF as shown in Table 1. According to Fuller (1989) probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller R., 1989). Lactobacilli and bifidobacteria are the most accepted microbes for probiotic application Isolauri E. et al., 1991; Saavedra JM. Et al., 1994; Isolauri E. et al., 1995; Engelbrektson A. et al., 2009; Sgouras DN. et al., 2005; Saggioro A. et al., 2005; Ruiz-Palacios G, Guerrero ML., Hilty M., 1996; Sellars RL., 2007; Millette M, Luquet, FM., Lacroix M., 2007; Mcfarland LV., 2006; Hilton E., 1997).

2. Conclusions

It has been concluded that it cannot be possible to prepared microbes free powdered infant formula due to its method of preparations. These microbiological contaminants are highly pathogenic and may cause severe infections which may sometimes leads to death of neonates and infants. Some bacteria may produce beneficiary. Although PIF are commonly used in developed and underdeveloped countries but the regulatory authorities of all over the world strongly advocate and forcefully recommend mother feed but in some scenario PIF is preferred over mother.

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