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MICROBES IN PEDIATRIC INFANT FORMULA

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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 life-threating 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.

Keywords: pathogenic bacteria; contamination; hazards; probiotics

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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 mother-feed (Fanaro S, Chierici R, Guerrini P, Vigi V, 2003). In normal vaginal delivery, an infant is exposed to the mother's vaginal 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 mother-fed 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. Mother-feeding rate is 95% and less than 30% in European and Scandinavian countries respectively. There is high rate of mother-feeding 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 bottle-fed 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 diarrhea-causing 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). HIV-positive 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 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 post-processing 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.

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).

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, non-toxic 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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