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Microbial Quality of Formulated Infant Milk Powders

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Abstract: Study was carried out to examine the microbiological quality of Infant formula milk powder. Total 60 of dried milk powders, 20 each of Group A (1-6), B (7-12) and C (13-18 months). Infant formula milk powders were purchased from Hyderabad, Sindh and evaluated for microbiological examination, like Total Viable Count (TVC), Enterobacteriaceae Count (EbC) and Yeasts and Moulds Count (YMC). Total viable count, ($3.4x10^3 \pm 5.0x10^2$cfu/g) were significantly higher than thermoduric count, ($<10 \pm 2.3x10^1$cfu/g) Thermophilic spores ($1.4x10 \pm 2.4x10^1$ cfu/g) Enterobacteriaceae count, (<5 ±1.0x10cfu/g) and Yeasts and Moulds (<5±1.0 cfu/g) respectively in all samples of infant formula of milk powders. Total Viable Counts group A, B and C having non significant difference. Even incase of thermoduric thermophilic spores, enterobacteriaceae, yeast and moulds were non significant recorded. The obtained averaged results compared to Indian Standard Institution (ISI) values. In Group A total viable counts were (12.18 folds), Group B (14.70 folds) and in Group C (15.15folds) lower than the ISI standard. thermoduric counts averaged compared with ISI standards and results were found lower in Group A (12.5 folds), Group B (16.0 folds) and Group C (14.2 folds) as compared to Indian Standards Institution, thermophilic spores in Group A (7.14 folds), Group B (6.6 folds) and Group C (6.25 folds) as compared to ISI. Enterobacteriaceae were lower than ISI standards (33 folds) in Group A, (33 folds) Group B and (25 folds) in Group C. Yeast and Moulds were lower than the ISI standards Group A (33 folds), Group B (33 folds) and in Group C (25 folds). Although Total Viable Count were within the range of standard of specification of (ISI) and the counts of thermophilic Spores, enterobacteriaceae counts and yeast and moulds also indicates the hygienic condition of Infant formula milk powders without risk level for human health.

Key words: Infant milk powder, enterobacteriaceae count, yeasts and moulds count

INTRODUCTION

Every parent heed about their health and feeding. The best source of babies feeding is their mother's milk (breast feeding). But in few cases the mother naturally fails to fulfill the breast feeding requirement of baby due to disease factor or hormonal imbalance. But in some cases the mother itself is not interested in breast feeding, reasons manifested, the working females can not provide proper feeding to their baby. In rare cases modern world females do not feed their babies just to maintain their apparently beauty. So they follow the infant formula (available in market) suggested by their nutritionist or doctor. No doubt, the Infant milk powders are generally considered as product of good microbiological quality with no risk of spoilage, but several factors may contribute to change its physical and chemical properties which reduce shelf-life and thus its commercial value (Cousins *et al*., 1987). Although the micro-organisms in infant milk cannot grow due to its low moisture content and do not play any direct role in their spoilage. But their occurrence in infant milk powder is of great significance and serves as an index of hygienic standards maintained during production, processing and handling. The infant milk provides a highly nutritious substrate that can support the wide variety of bacteria as well as yeast and molds for their growth and reproduction (Phillips and Griffiths, 1990). The contamination role of bacteria during the preparation of infant milk powder has been well documented. The thermophilic can have significant economic consequences when they exceed specification limits and may result in down grading of the products (Ronimus *et al*., 2005). Because these have ability to produce extremely heat resistant spores, and thus are significant source of pre- and post pasteurization (White *et al*., 1993). Since, no work has been reported on any aspects of Infant milk powders in the province of Sindh. Thus, present study has been designed for evaluating microbiological quality of milk powders.

MATERIALS AND METHODS

Collection of infant powder samples: A total of sixty samples of infant milk powders i.e 20 form each category A (1-6), B (7-12) and C for (7-18 months) babies were purchased from Hyderabad and brought to

the Laboratory of Dairy Microbiology, Department of Dairy Technology. Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, for microbial examination.

Preparation of test samples: Milk powder (10 g) was diluted in warm (45°C) sterile diluents peptone water solution (90 ml) to make primary dilution (10^{-1}). Then a series up to 10^{-5} dilution was prepared by transferring primary dilution (1 ml) into test tube containing sterile diluents (9 ml) to obtain 10^{-2} dilution and repeating the operations with sterile diluents (9 ml) using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4} and/or 10^{-5}.

Enumeration of total viable count (Colony count technique at 30°C): Total viable counts were enumerated according to the method of International Dairy Federation (IDF, 1991). Pre prepared test sample (1 ml) of 10^{-3}, 10^{-4} and/or 10^{-5} dilutions (section-3.9.1) was transferred into sterile petri dishes in duplicate through sterile graduate pipette and/or dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile plate count agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (30°C) for 72±2 h. Parallel to that, control plates were also prepared using similar medium (15 ml) to check the sterility. The dishes containing more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The result was calculated using following formula.

Enumeration of Enterobacteriaceae counts (Colony count technique at 37°C): Enterobacteriaceae counts were enumerated according to the method of British Standard Institute (BSI, 1993). Pre prepared test sample (1ml) of 10^{-1}, 10^{-2} and/or 10^{-3} dilution (section 3.9.1) was transferred into sterile petri dishes through dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile violet red bile agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (37°C) for 24±2 h. Parallel to that control plates were also prepared using similar medium (15 ml) to cheek its sterility. The dishes containing more than 10 and/or fewer than 200 colonies were selected and counted using colony counter. The result was calculated using formula as mentioned in section 3.10.

Enumeration of yeasts and moulds counts (Colony count technique at 25°C): Yeasts and moulds count were enumerated according to the method of IDF (1990). Pre prepared test sample (1 ml) of 10^{-1}, 10^{-2} and/or 10^{-3} dilution (section-3.9.1) was transferred into sterile petri dishes through dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile potato dextrose agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (25°C) for 5 days. Parallel to that control plates were also prepared using medium (15 ml) to cheek the sterility. The dishes containing more than 10 and/or fewer than 150 colonies were selected and counted using colony counter.

Enumeration of thermoduric and thermophilic spore counts (Colony count technique at 55°C): Thermoduric and thermophilic count was enumerated according to the method of Marshall (1993). Milk powder (10 g) was reconstituted in peptone water diluents (90 ml) and heated (80°C or 100°C) for 10 or 30 minsts to eliminate the vegetative cells. Heat treated sample (1 ml) of 10^{-1}, 10^{-2} and/or 10^{-3} dilution was transferred into petri dishes (in duplicate) through sterile pipette automatic pipette (1000 µl) and warm (45±1°C) sterile nutrient or milk starch agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (55°C) for 48 h. Parallel to that control plates were also prepared using medium (15 ml) to cheek the sterility. The dishes containing more than 10 and/or fewer than 200 colonies were selected and counted using colony counter.

Total viable count: Total viable count of Group A (1-6 months), Group B (7-12 months) and Group C (13-18 months) was evaluated and the results are presented in Fig. 1. No wide variation was observed in TV counts in all types of infant powders were examined in the present study. The concentration of TV count in Group A, ranged between $2.3x10^3$ to $4.8x10^3$ cfu/g and averaged $3.9x10^3 \pm 3.0x10^2$ cfu/g. While in case of Group B, the TV counts were observed in between $1.5x10^3$ to $5.3x10^3$ cfu/g with mean value of $3.4x10^3 \pm 5.0x10^2$ cfu/g. Where ever, TV count in Group C, varied between $1.2x10^3$ to $6.5x10^3$ cfu/g and averaged $3.3x10^2 \pm 6.1x10^2$ cfu/g. Moreover, the results of statistical analysis showed no significant difference ($p>0.05$), in TV counts in Group A, Group B and Group C. The concentration of TV counts were lower in Group A (12.18 folds), Group B (14.70 folds) and in Group C (15.15 folds) compared to Indian Standards Institute, ISI and Pakistan Standard Institution i-e $\leq 5.0x10^4$ cfu/g.

Thermoduric count: Thermoduric count of (Group A), (Group B) and (Group C) was evaluated and the results are presented in Fig. 2. A wide variation was not observed in TD counts in all types of infant formula powders examined in the present study. The concentration of TD count in Group A ranged between 10x0 to 2x10 cfu/g and averaged <10 and >5±$2.3x10^1$ cfu/g. While in case of Group B, the TD counts were observed in between 10x0 to 1.1x10 cfu/g with mean value of <10 and >5±$1.4x10^1$ cfu/g. Where ever, TD count in Group C varied between 1x0 to 1.2x10 cfu/g and averaged <10 and >5±$1.5x10^1$ cfu/g.

Table 1: Total Viable Counts (cfu /g) in different infant formula samples compared to ISI/PSI standards.

	Total Viable Count (TVC) cfu/g	
Sample	Observed (a)	Deviation in folds from ISI standard (b) = (x) ÷ (a)
Group A	3900	-12.8
Group B	3400	-14.70
Group C	3300	-15.15

a = Observed Values
x = (Standard Value of PSI/ISI = ≤ 50000 cfu/g)
ISI = Indian Standards Institution

Table 2: Thermoduric Counts (cfu /g) in different infant formula samples compared to ISI standards.

	Thermoduric Count (TDC, (cfu/g)	
Sample	Observed (a)	Deviation in folds from ISI standard (b) = (x) ÷ (a)
Group A	≤10	-10
Group B	≤10	-10
Group C	≤10	-10

a = Observed Values
x = (Standard Value of ISI (1993) = ≤$1.0x10^2$ cfu/g)
ISI = Indian Standards Institution

Table 3: Thermophilic Counts (cfu /g) in different infant formula samples compared to ISI standards.

	Thermophilic Spore Count (TPSC cfu/g)	
Sample	Observed (a)	Deviation in folds from ISI standard (b) = (x) ÷ (a)
Group A	14	-7.14
Group B	15	-6.6
Group C	16	-6.25

a = Observed Values
x = (Standard Value of ISI (1993) = ≤$1.0x10^2$ cfu/g)
ISI = Indian Standards Institution

Furthermore, Analysis of Variance (ANOVA) showed significant difference ($p<0.05$), in TD counts in Group A, Group B and Group C. It was further observed that TD count of Group A, Group B and Group C was no significantly different ($p>0.05$) in TD counts observed among all groups. The concentration of TD counts was lower in Group A (12.5 folds), Group B (16.0 folds) and in Group C (14.2 folds) compared to that of Indian Standards Institute (ISI, 1975) i-e ≤ 1.0x10 cfu/g (Table 2).

Thermophilic spore count: Group A, Group B and Group C were evaluated for thermophilic spore count and the results are presented in Fig. 3. No variation was observed in TPS counts in all groups of infant formula milk powders examined in the present study. The concentration of TPS count in Group A ranged between <5 to 2.7x10 cfu/g and averaged 1.4x10 ±2.4x10 cfu/g. While in case of Group B, the TPS counts were observed in between <5 to 2.3x10 and averaged $1.5x10^2$±$2.2x10^1$ cfu/g. TPS count in FCMP varied between <10 to 2.3x10 cfu/g and averaged 1.6x10±2x10 cfu/g.

Furthermore, the results of statistical analysis (AOV) showed non significant difference ($p>0.05$), in TPS

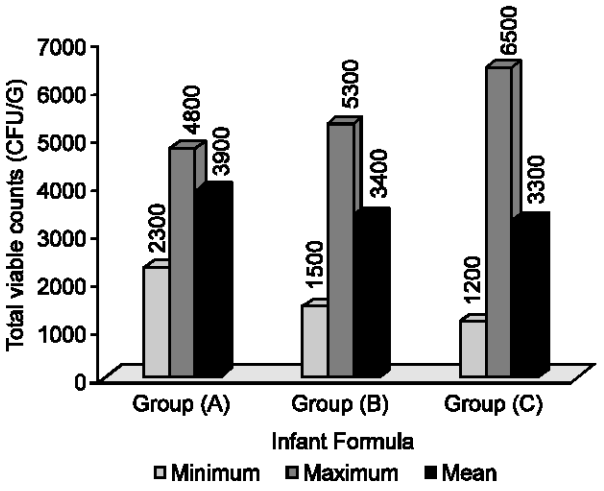

Fig. 1: Graph shows minimum, maximum and mean values of total viable counts (cfu/g) in infant formula

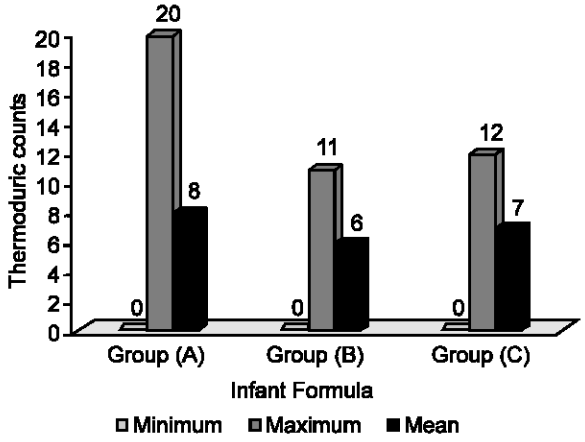

Fig. 2: Graph shows minimum, maximum and mean values of Thermoduric counts (cfu/g) in infant formula

counts in Group A, Group B and Group C. The concentration of TPS counts was lower in Group A (7.14 folds), Group B (6.6 folds) and in Group C (6.25 folds) compared to that of Indian Standards Institute (ISI, 1975) i-e ≤ $1.0x10^2$ cfu/g.

Enterobacteriaceae count: All the three groups were evaluated for Enterobacteriaceae count and the results are depicted in Fig. 4. TPS counts did not vary greatly in all types of infant formula of milk powders examined in the present study. The concentration of Enterobacteriaceae count in Group A ranged between 10x0 to <10 and >5 cfu/g and averaged <5 ±1.0x10cfu/g. While in case of Group B the Enterobacteriaceae counts were observed in between 10x0 to <10 and averaged <5 ±1.0x10 cfu/g. whereas in Group C, varied between <5 to <10 cfu/g and averaged <5 ±1.0x10cfu/g.

Table 4: Enterobacteracea Counts (cfu /g) in different infant formula samples compared to ISI standards.

	Enterobacteraceae Count (EbC cfu/g)	
Sample	Observed (a)	Deviation in folds from ISI standard (b) ÷ (a)
Group A	≤5	-20
Group B	≤5	-20
Group C	≤5	-20

a = Observed Values
x = (Standard Value of ISI (1993) = ≤1.0x10^2 cfu/g)
ISI = Indian Standards Institution

Table 5: Yeast and Mold Counts (cfu /g) in different infant formula samples compared to ISI standards.

	Yeasts and Moulds Count (YMC cfu/g)	
Sample	Observed (a)	Deviation in folds from ISI standard (b) = (x) ÷ (a)
Group A	≤5	-20
Group B	≤5	-20
Group C	≤5	-20

a = Observed Values
x = (Standard Value of ISI (1993) = ≤1.0x10^2 cfu/g)
ISI = Indian Standards Institution

Moreover, the results of statistical analysis (AOV) showed non significant difference ($p>0.05$), in Enterobacteriaceae counts in Group A, Group B and Group C The concentration of Enterobacteriaceae counts was higher in Group A (33 folds), Group B (33 folds) and in Group C (25 folds) compared to that of Indian Standards Institute (ISI, 1993) i-e ≤ 1.0x10^2 cfu/g.

Yeasts and moulds count: Yeasts and moulds count of Group A, Group B and Group C was examined and the results are shown in Fig. 5. It was observed that Yeast and Moulds counts in all types of infant milk powders did not show variation. However, the concentration of yeasts and moulds count in Group A ranged between 10x0 to <10 cfu/g and averaged <5 cfu/g. While in case of the Group B moulds counts were observed in between 10x0 to <10 and averaged <5 ±.1.0 cfu/g, whereas in Group C, varied between <5 to <10 cfu/g and averaged <5±1.0 cfu/g.

Statistical analysis (AOV) revealed non significant difference ($p>0.05$) in yeasts and moulds counts in Group A, Group B and Group C. The concentration of Yeast and Moulds counts were lower in Group A (33 folds), Group B (33 folds) and in Group C (25 folds) compared to that of Indian Standards Institute (ISI, 1975) i-e ≤ 1.0x10^2 cfu/g.

RESULTS AND DISCUSSION

Present study has been conducted to assess the general hygienic quality of Infant milk powders and the extent of microbes has been observed. Although the microorganisms in infant formula milk owing to their low moisture content can not grow and thus do not play any direct role in their spoilage, their occurrence in these

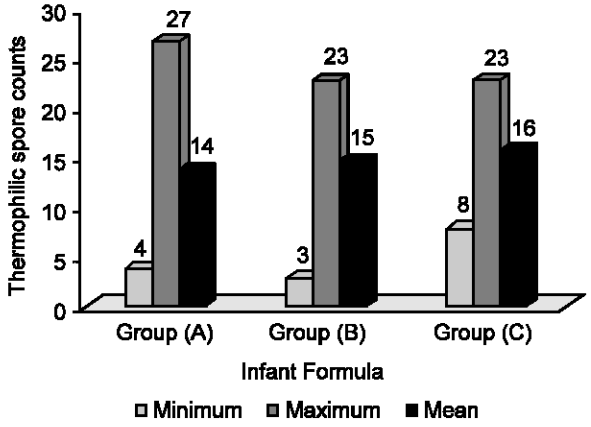

Fig. 3: Graph shows minimum, maximum and mean values of Thermophilic spore counts (cfu/g) in infant formula

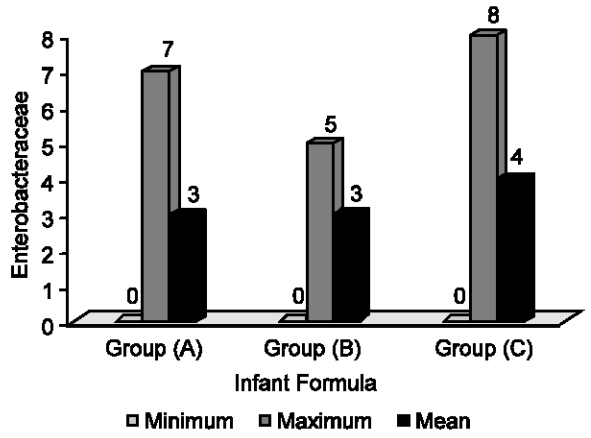

Fig. 4: Graph shows minimum, maximum and mean values of Enterobacteraceae counts (cfu/g) in infant formula

products is of great significance they server as an index of hygienic standards maintained during Production, Processing and handling (Yadav *et al*., 1993). In the present study the total viable count of Group A, (3.9x10^3±3.0x10^2 cfu/g) was not significantly ($p>0.05$) higher than Group B, (3.4x10^3±5.0x10^2 cfu/g) and Group C (3.3x10^2 ±6.1x10^2 cfu/g).

It is of interest to point out that total viable count of all three groups (3.9x10^3±3.0x10^2 cfu/g), (3.4x10^3±5.0x10^2 cfu/g), (3.3x10^2 ±6.1x10^2 cfu/g) observed in the infant formula is lower than reported by Rueckert *et al.* (2005); Khaskheli (1998); ISI (1975) and PSI (2007) i.e 5.6x10^4±4.3x10^3, 8.7x10^3, 1.0x10^4 and 5.0x10^4 respectively. The vegetative cells normally killed at 80°C for 10 min. But the present study manifested their presence in infant powders. Because, their ability to attach with stainless steel and folded surface (Flint *et al*., 2006). Once they attach to the surface, vegetative cells

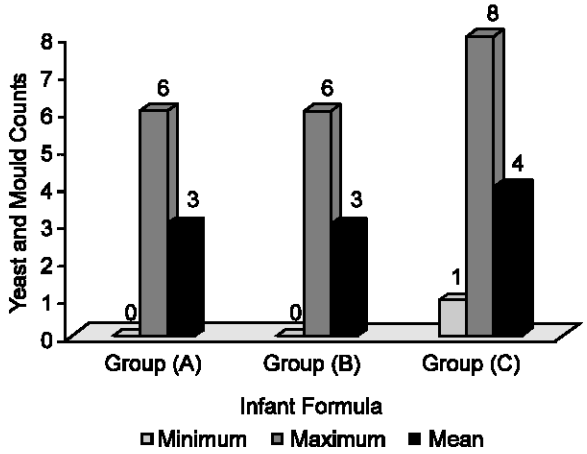

Fig. 5: Graph shows minimum, maximum and mean values of Yeasts and Molds counts (cfu/g) in infant formula

grow with spores by forming biofilms. This biofilms is not completely removable by CIP system but can be decreased; the remaining contaminants present on folded stainless steel transfer into final product, i.e powders milk (Parker *et al*., 2001).

Thermoduric count of Group A, (<10 and >5±2.3x10^1 cfu/g) was not significantly (p>0.05) higher than Group B (<10 and >5±1.4x10^1 cfu/g) and C (<10 and >5±1.5x10^1 cfu/g). Moreover, the mean (3.7x10^1) of TD counts in the present study is lower than reported values (1.8x10^1 cfu/g) of Infant milk powders. (3.8x10^1cfu/g), Ronimus *et al*. (2005). The reason of thermoduric growth is processing, if they are present in raw milk their growth accelerate at the time of pasteurization, because temperature of pasteurization is favorable for the growth of thermoduric bacteria (Murphy *et al*., 1999). The transit time between the silo milk and spray drier is typically 20-30 min there is obviously bacterial growth is clearly associated with processing and bio transfer to the end product (Flint *et al*., 2006; Wirtanen *et al*., 1996 and Stadhouders *et al*., 1982).

The thermophilic spore of Group A (1.4x10±2.4x10^1 cfu/g) was lower than Group B (1.5x10^2±2.2x10^1 cfu/g) Group C (1.6x10±2x10 cfu/g) However, the averages obtained in present study is lower than the mean value reported by Rueckert *et al*. (2005) i.e (3.2x10^4±3.4x10^3 cfu/g) and (2.4x10^4±5.1x10^3 cfu/g). If the spores are present in raw milk that rapidly grow, when they obtain favorable temperature during milk processing (pasteurization), the other evidence provided i.e foulant, it is a major source of thermophilic contamination in a full scale milk powder plant (Scott *et al*., 2007).

Enterobacteriaceae count of Group A (<5±1.0x10cfu/g) was not significantly (p>0.05) higher than Group B (<5 ±1.0x10 cfu/g) and Group C (<5±1.0x10cfu/g). The mean values of all three groups A, B and C obtained in present study is lower than reported by Taha *et al*. (1972) i.e 13x10^6. It is the general concept that enterobacteriaceae are not present in Infant formula proved by various researches. The Infant formula powder is packed hygienically in large sterilized containers and bags. However, transportation some damaged containers and bags have been observed, probably they can contaminate the milk powders.

The yeasts and moulds count of Group A (<5 cfu/g), Group B (<5±.1.0 cfu/g) and Group C (<5±.1.0 cfu/g) were non significant different. However, the mean value (3.6x10^2±3.8x10^1) of yeasts and moulds in the present study is lower than the results presented by Rossi *et al*. (1974) i.e >100000/100 g in powder milk and reported by Ceittao *et al*. (1973) i.e <1000/g in milk powder. Presence of yeasts and moulds in milk or milk products, molds may create hazard to one's health, produce an allergen and an irritant to human health (Parihar and Parihar, 2008).

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