

Original article

Microbial load and microflora of weaning foods obtained from pediatric outpatients in Addis Ababa.

Wolde-Aregay Erku¹, Mogessie Ashenafi²

Abstract: One hundred samples of feeding bottle contents from outpatient infants visiting four public clinics in Addis Ababa were analyzed for their microbial load and microflora. Forty-nine percent of the infants were female. The educational status of the mothers was illiterate (21%), elementary-level education (27%), and high-school level education and above (52%). Ingredients of the feeding bottles consisted of six different types and about 60% of the studied infants consumed cow's milk. Over 50% of the mothers used the same bottle throughout the day to feed the child and only 9% used three or more bottles interchangeably. High levels of bacterial contamination were observed in all samples with counts ranging between 1.6×10^5 and 9.9×10^8 cfu/ml of bottle content. Cow's milk and cereal gruel were the most heavily contaminated ones. A total of 366 bacterial strains belonging to 12 genera were isolated from feeding bottle contents. The dominant isolates were coliforms (31.2%), followed by staphylococci (30.1%), *Bacillus* spp. (19.1%), and micrococci (14.2%). Of the factory-produced weaning food samples (30), only nine were contaminated with over 10^2 cfu/g or ml. Possible sources of high contamination could be poorly cleaned and frequently re-used utensils, contamination during refilling and feeding bottles themselves. Findings of this study indicated the need for educating mothers on hygienic handling of infant food. The importance of thorough cleaning of feeding bottles and avoiding post-cooking contamination during storing the cooked foods should be stressed. [*Ethiop. J. Health Dev.* 1998;12(2):141-147]

Introduction

Children constitute about 31.7% of world population and over 44% of the African population (1,2). About 46% of the Ethiopian population consists of children aged ≤ 14 years (3). Nevertheless, estimates show that the cumulative risk of dying before the age of five years for Ethiopian infants and children is 220 per 1000 live births (2) and the death rate of Ethiopian infants under five months of age is 293 per 1000 (4). Child morbidity is also a major global problem, especially in the developing countries. One of the most common causes of child morbidity in these countries is diarrhoea due to various food- and water-borne pathogens. Though the sanitary conditions in which the infants are found have important role in the acquisition of these pathogens (5), it is by far the weaning foods and the way they are handled and fed that play the significant role.

It is an acceptable practice that when the infant reaches 4 - 6 months of age, breast milk must be supplemented, and later replaced by appropriate foods. However, with the introduction of

¹From the Department of Biology, Addis Ababa University, P.O.Box 1176, Addis Ababa,

²Department of Immunology and Microbiology, Institute of Pathobiology, Addis Ababa University, P.O.Box 1176, Addis Ababa.

weaning foods, which in many countries are prepared under unhygienic conditions, the risk of getting foodborne diarrhoeal pathogens by the infants is increased (6). The incidence of diarrhoea is highest at the second six months of life as this is the age which coincides with introduction of weaning foods (7,8,9) Brown *et al.*(7) and Popkin *et al.* (10) revealed that the addition of even water, tea, and other non-nutritive liquids to breast-milk doubled or tripled the likelihood of diarrhoea; with supplementation of additional nutritive foods or liquids, the risk of diarrhoea further increased significantly.

In Ethiopia, infants are fed with various types of weaning foods depending on the income of the family. In a city-wide household survey in Addis Ababa, Ketsela and Kebede (11) found out that about 44% of infants were bottle-fed. According to the manner the weaning foods are handled after preparation, various types of microorganisms are likely to be introduced into them, and may further proliferate. Wolde-Tensay and Tesfaye (12) isolated more than 13 enteric bacterial pathogens from feeding bottles in Addis Ababa.

Bottle-feeding is the most important route of exposure of infants to diarrhoeal pathogens. Jellife and Jellife (13) emphasized that diarrhoeal diseases due to enteric infections are the main killers of bottle-fed babies. Studies conducted in Ethiopia regarding bottle-feeding showed similar results (14).

The objective of the study was, thus, to determine how exposed infants are infected by contaminated weaning foods. The microbial load of ready-to-consume feeding bottle contents as made available to the infant/child and the microbial status of locally manufactured weaning foods were assessed. The relation between bacterial contamination of weaning foods and the ingredients on one side and the level of education of mothers on the other was also determined.

Methods

Sample collection: One hundred samples of bottle content were collected from outpatient infants who visited four public clinics in Addis Ababa. These were Kirkos Clinic, Beletshachew Clinic, Higher 18 Clinic, and Lidetta Clinic. Each bottle content was shaken thoroughly and 20 ml of it was transferred into sterile screw-capped test tube. The samples were then taken to the Microbiology Laboratory of the Biology Department, Addis Ababa University, for bacteriological analyses within two to three hours.

The following information was also collected by interviewing the persons who brought the infants to the clinics: age and sex of the infants, constituents of the bottle content, volume (in bottles) the infant was fed during the day, and educational level of the mother or the person who was looking after the infant.

Preparation of Weaning Foods from Manufactured Products and Home-made Ingredients: A total of 30 samples of weaning foods were prepared from locally manufactured and packed weaning foods (products A, B and C). Products A and C were cereal-based infant foods mixed with powdered milk, and product B was dehydrated milk. The preparation was conducted by experienced mothers at home according to the instructions of the manufacturers. About 20 ml of ready-to-consume preparations of products A and C were transferred into screw-capped test tubes, and about 25 g of product B was transferred into a sterile plastic bag. All of the samples were taken to the laboratory for bacteriological analyses within two to three hours. Similarly, of the commonly encountered home-made weaning foods (cow's milk and thin gruel made of cereal blend), 10 samples were prepared at household level, collected aseptically and microbiologically analyzed.

Isolation and characterization of microflora: Samples (10 ml or gram) of the foods were homogenized in 90 ml of physiological saline solution, and subsequently diluted. Appropriate dilutions (0.1 ml) were spread-plated onto the following media: Tryptone Glucose Yeast Agar (TGYA) (Oxoid) for aerobic mesophilic count, Mannitol Salt Agar (MSA) (Oxoid) for isolation and enumeration of staphylococci, and MacConkey agar No. 3 (Oxoid) for isolation and enumeration of coliforms. Incubation was at 32°C for 24-36 hours.

After counting aerobic mesophilic bacteria, ten colonies were picked from each countable plate, further purified by repeated plating and characterized to the genus level using the following tests.

Cell shape, cell grouping pattern, and gram reaction were determined microscopically after gram staining. The production of endospore was according to Claus and Berkeley (15). Motility was determined by inoculating a 24-hour culture into sulfur Indole Motility (SIM) medium (Oxoid) and incubating for 24 hours at 37°C.

Presence of cytochrome oxidase was tested by the method of Kovacs (16), and catalase test was performed by flooding young cultures with 10% H₂O₂ solution. Production of indole was tested by putting few drops of Enrich's reagent (p-dimethyl aminobenzaldehyd, 1 g; concentrated HCL, 20 ml;

and absolute ethanol, 95 ml) into culture grown in tubes of SIM medium (Oxoid). Methyl red reduction and acetyl methyl carbinol production was tested using MRVP medium (Oxoid). Utilization of citrate as sole source of carbon was tested on Simmon's citrate medium (Oxoid) and Oxidation / Fermentation test for Gram negative rods on Hugh and Leifson O/F medium as described by Collins and Lyne (17). For Gram positive cocci, Baired-Parker's modification of Hugh and Leifson medium was used as recommended by Collins and Lyne (17).

The pattern of carbohydrate utilization was studied on glucose, lactose, sucrose, mannitol, dulcitol, salicine and xylose. Hydrolysis of starch was tested on nutrient agar containing 1% soluble starch.

Results and Discussion

Of the 100 infants and children considered in the study, 51 were males and 49 females. Twenty-nine of the males and 19 of the females were ≤ 6 months of age; and 16 of the males and 21 of the females were between 7 and 12 months old. Only six of the males and nine of the females were over the age of one year

The educational status of the mothers was variable with 21 of them being illiterate, 27 having elementary level education, and 52 high - school education and above. Of the 51 infants who were fed with the same bottle throughout the day, 32 were < 6 months old, 13 were between 7 and 12 months old, and six were >12 months old.

Table 1: **Type of bottle content and number of bottles used in relation to educational level of mothers.**

Educational status of mothers	Ingredients of bottle contents							No. bottles used/day		
	No.	I	II	III	IV	V	VI	1	2	≥3
Illiterate	21	11	1	3	2	-	4	16	3	2
Elementary level	27	18	-	6	1	-	2	18	8	1
High school level and above	52	31	2	5	2	5	4	17	29	6
Total	100	30	3	14	5	5	13	51	40	9

I, cow's milk; II, powdered milk III, cereal blend IV, milk + cereal
 V, cereal + legume VI, others

The ingredients of the feeding bottles were categorized into six groups: cow's milk, commercial powdered milk, gruel made from cereal blend, gruel made from mixture of cereal and milk, gruel made from mixture of cereals and legumes (locally known as *mitin*), and other items such as tea, *abish*, and water regained after cooking rice, etc. The large proportion of the studied infants consumed cow's milk (60%) followed by cereal blend (14%). Of the 48 infants under the age of seven months, 43 consumed cow's milk. Of the 37 infants aged between 7 to 12 months, 11 were fed only with cow's milk and so were all infants aged >12 months.

Over half of the mothers (51%) considered in this study used the same bottle throughout the day in feeding their child (Table 1). This consisted of 67% of the illiterate and elementary level educated mothers, and 33%

Table 2: **Total bacterial load in relation to educational level of mothers and bottle content**

count(cfu/ml)	Educational status of mothers	Ingredient of bottle						Total
		I	II	III	IV	V	VU	
10 ⁵ - 10 ⁶	Illiterate	2	-	-	-	-	-	2
	Elementary	4	-	-	-	-	-	4
	High school and above	8	-	-	-	2	-	11
>10 ⁶ - 10 ⁷	Illiterate	3	-	-	-	-	1	4
	Elementary	4	-	-	-	-	3	7
	High school and above	8	-	1	-	-	3	12
>10 ⁷ - 10 ⁸	Illiterate	3	1	1	1	-	1	7

	Elementary	3	-	2	-	-	-	5
	High school and above	9	-	1	-	2	3	15
>10 ⁸	Illiterate	3	-	3	1	-	1	8
	Elementary	7	-	3	1	-	-	11
	High school and above	6	2	3	1	1	1	14
	College	-	-	-	-	-	-	-
Total		60	3	14	5	5	13	100

I, cow's milk; V, cereal + legume II, powdered milk VI, others III, cereal blend IV, milk + cereal

of mothers educated at high school level or above. Only 9% of all mothers interchangeably used three or more bottles, while 40% of the mothers used two bottles. This is in contrast to the finding of Ilegbe *et al.* (18) who reported that 78% of educated mothers they studied used at least three bottles. The most preferred infant food in this study was cow's milk. Sixty percent of all mothers and over half of the mothers at every level of educational status fed their infants with cow's milk. Almost all mothers at the various educational status used cereal blend as a second choice to feed their infants. It is interesting to note that the cereal and legume combination, which is supposed to be nutritious with respect to its protein content, was considered only by mothers with high school education or above. The fact that this combination was not considered by illiterate mothers and mothers with elementary level education is also remarkable as such mothers, in most cases, belong to low income families which should normally consider the use of a less expensive but also nutritious food.

In the majority of the cases educational status of the mothers seemed important in determining the number of bottles used. For example, the data showed that less than a quarter of the illiterate mothers used two or more bottles, whereas 67% of mothers with high school education or above used two or more bottles in a day to feed their infants. This may be attributed either to lack of appropriate knowledge or financial capability of the illiterate mothers.

Table 3: Bacterial load in relation to interchangeable number of feeding bottles

Number of inter changeable bottles	Bacterial count (c.f.u./ml)				Total
	0 ⁵ - 10 ⁶	>10 ⁶ - 10 ⁷	>10 ⁷ - 10 ⁸	>10 ⁸	
1	7	12	14	18	51
2	8	10	11	11	40
3	2	1	2	4	9
Total	17	23	27	33	100

A fairly high level of bacterial contamination was observed in all samples with counts ranging between 1.6 x 10⁵ c.f.u./ml, and 9.9 x 10⁸ c.f.u./ml of bottle content. Over 80% of the samples had counts > 10⁶ c.f.u./ml and of these, 33% yielded counts > 1.0 x 10⁸ c.f.u./ml. These figures are very high when compared to results of other studies conducted elsewhere. Imong *et al.*(19) reported a mean total bacterial count of 3.8

Table 4: Frequency distribution of dominant genera isolated from the various weaning foods

Food types	No of isolates	Dominant isolates*						
		i	iii	iii	iv	v	vi	vii
Cow's milk	217	17	55	3	1	67	32	42
Milk powde	11	1	2	-	-	4	2	2

Cereal blen	59	7	16	3	-	15	7	11
Milk + cereal	11	2	4	-	-	2	1	2
cereal + legum	23	1	5	-	1	5	4	7
others	45	2	13	-	1	17	6	6
Total	366	30	95	6	3	110	52	70

*i, *E. coli* ii, other coliforms iii, *Proteus* spp. iv, *Alcaligenes* spp. v, *Staphylococci* vi, *Micrococci* vii, *Bacillus* spp.

$\times 10^4$ with only 10% having counts $> 10^6$ organisms/g. These authors claimed that the figures they reported were above internationally recommended “safe” levels. A community-based microbiological study made in a rural Ethiopian setting showed that over 50% of the samples had counts between 10^6 and 10^7 cfu/ml (20).

The two most commonly consumed bottle contents (cow’s milk and cereal gruel) were the most heavily contaminated (Table 2). Only 14 of the 60 samples of cow’s milk gave counts $\leq 10^6$ c.f.u./ml or below; and over half of the cow’s milk samples contained $\geq 10^7$ c.f.u./ml. Cereal blend foods (12 of 14) also had similar levels of high contamination. Such high gross bacterial contamination of milk and cereal - based weaning foods was also noted by Black *et al.* (21), when they investigated etiology of infantile diarrhoea in Bangladesh.

Seventy one percent of the samples from infants of illiterate mothers contained count of more than 10^7 c.f.u./ml. Over 40% of bottle contents from infants with mothers educated at high school level or above had counts between 10^5 and 10^7 c.f.u./ml. Although this count was relatively lower than that observed for mothers with lower level education, it was beyond the acceptable load for fluid foods where bacterial proliferation is fast. Findings of other studies of weaning foods and feeding bottles showed that the degree of bacterial contamination decreased with increased educational level of the mothers (18,22).

Of the 51 mothers who used single feeding bottle, 32 had bottle content counts of more than 10^7 c.f.u./ml (Table 3). Similar counts were also noted for mothers who used two feeding bottles (22/40) and three bottles (6/9). Although frequent changing of feeding bottles should help to lower the microbial load of the weaning foods, the observation in this study showed very little difference. This may be due to the possibility that the weaning foods were stored in a larger container before being dispensed in the feeding bottles. If bacterial proliferation took place during storage, frequent changing of feeding bottles might not have any significant positive effect.

A total of 366 bacterial strains belonging to 12 genera were isolated from the feeding bottle contents considered in this study (Table 4). The dominant isolates were coliforms (34.2%) followed by staphylococci (30.1%), *Bacillus* spp. (19.1%), and *Micrococcus* spp. (14.2%). Although dominance of coliforms among the microflora of weaning foods in this study was quite high, it was much lower than the 77.8% reported for weaning foods from Addis Ababa (12). High coliform incidence in weaning foods were also noted by Motarjami (9) elsewhere. Total coliform count of weaning foods was reported to be related with food hygiene practices and maternal factors (22). The coliforms in this study were dominated by *Klebsiella* spp. (44%). *E. coli* also made up 22% of the coliforms, and this was similar to the 23% reported in Addis Ababa (12), thus indicating similar levels of faecal contamination. This, however, was much lower than the 31.3% reported by Odugbemi *et al.*(23) for *ogi*, a fermented cereal weaning food in Nigeria, and the 72.3% reported for

Table 5: Microbial load of factory-produced and home made weaning foods as prepared at household level.

Sample source	sample	No. of samples	count (c.f.u./ml)				
			$<10^2$	10^2	10^3	10^4	10^5
	A	10	7	2	1	-	-
Factory-produced	B	10	4	3	3	-	-
	C	10	7		-	1	2
Home-made	I	10	9	1	-	-	-

	11	10	8	1	1	-	-
Total	50	35	7	5	1	1	

samples of weaning foods and other sources of contaminations in India (24).

Of all the bacterial strains isolated in this study, 59% were isolated from cow's milk and 16% from cereal gruel. Since these food types are normally cooked thoroughly, they are not expected to yield faecal coliforms which are killed at the cooking temperatures. Thus post-cooking contaminations are important factors that play a role in the hygienic quality of the weaning foods.

Only nine of the 30 factory-produced samples were found to be contaminated with over 10^2 c.f.u./ml or g. The rest had count of below 10^2 c.f.u. /ml or g. The flora was mainly dominated by aerobic spore-formers. Three of the 10 samples from Product C yielded counts between 10^4 and 10^5 c.f.u./ml. The factory products are generally of good microbiological quality, although the relatively higher counts in product C may call for strict follow up of good manufacturing practices.

Home-made weaning foods (cereal blend, and cow's milk) prepared at house-hold level by mothers, under close inspection, had low levels of bacterial contamination. The microflora consisted of *Bacillus* species (Table 5). The fact that only *Bacillus* species were isolated is a good indication of the effectiveness of cooking in reducing bacterial contamination. A comparison between counts of freshly prepared weaning foods and those of the bottle contents clearly show that post-cooking contamination from various sources is the important factor in the poor hygienic quality of the bottle contents considered in this study.

The possible sources of high contamination observed in feeding bottle contents could be poorly cleaned and frequently reused utensils, contamination during refilling bottles, and the feeding bottles themselves. This notion is supported by reports of other workers (18,19). The investigation of Ilegbe et al. (18) on isolation of bacteria from feeding teats showed that 80% of the teats studied were grossly contaminated with bacterial pathogens. In a study of contaminants of weaning foods in low income groups in India, Ghuliani and Kaul (24) indicated that sources of contamination could be water used for cleaning feeding bottles, bottle nipples, mother's nails, utensils, mother's teats and child's hands.

Findings of this study indicated the need for educating mothers on hygienic handling of infant food. The importance of thorough cleaning of feeding bottles and avoiding post-cooking contamination during storing the cooked foods should be stressed.

Acknowledgements

The financial assistance for WE by SAREC is acknowledged.

References

1. World Health Organization. Integrated Management of the Sick Child. Bull Wld Hlth. Org 1995;73:735- 740.
2. World Health Organization. Demographic Data. Wld. Hlth Statist. Ann 1994. A.3 - A.8.
3. World Health Organization. Vital Statistics and Life Tables, Table c-1 Wld. Hlth. Statist Ann. 1992. C-3.
4. Shamebo D, Sandstorm A, Muhe L, Freijic L, Krantz I, Lonnberg G, Wall S. The Butajira Project in Ethiopia: a nested case - reference study of under - five mortality and its public health determinants. Bull Wld Hlth Org 1993;71:389
5. Baltzar JC, Tigola TV, Temponko SB. Hygiene Behavior and Hospitalised severe childhood Diarrhoea: a case - control study. Bull Wld Hlth Org. 1993;71:323-328.
6. World Health Organization. Research On Improved Infant Feeding Practices to Prevent Diarrhoea or Reduce its Severity: Memorandum from a JHU/WHO Meeting. Bull Wld Hlth Org. 1989;67:27-33.

7. Brown KH, Black RE, de Romano GL, de Kanashiro HC. Infant-feeding Practices and their Relationship with Diarrhoeal and other Diseases in Hauscar (Lima), Peru. *Pediatr* 1989;83:31-40.
8. Burn C, Martinez D, Glass I. The magnitude of global problem of diarrhoeal diseases: a ten year update. *Bull Wld Hlth Org.* 1992;70:705-714.
9. Motarjemi Y, Kaferstein F, Moy G, Quevedo F. Contaminated Weaning Food: a major risk factor for diarrhoea and associated malnutrition. *Reviews / Analyses. Bull Wld Hlth Org.* 1993;71:79-92.
10. Popkin BM, Adiar L, Akin JS, Black R, Briscoe J, Flieger W. Breast - feeding and Diarrhoeal Morbidity. *Pediatr* 1996;86:874-882.
11. Ketsela T, Kebede D. Pattern of feeding of infants in Addis Ababa, Ethiopia. *Ethiop J Hlth Dev.* 1996;10:57-65.
12. Wolde-Tensay Z, Tesfaye H. Isolation of Enteric Pathogens and Coliform Bacteria From Infant Feeding Bottle Content in Addis Ababa, Ethiopia. *Ethiop J Hlth Dev.* 1992;6:1-4.
13. Jelliffe DB, Jelliffe EF. Feeding Utensils and Infant Diarrhoea in Less Developed countries . *Am. J. Dis. Child* 1982;136:667.
14. Okbagzhi G, Gedlu E, G. Medhin E, Tsegaye E, Abdi F, Baye M. Infant Feeding Practices in Debre Tabor. In *Gondar College of Medical Sciences IXth Public Health and Research Conference.*
Ed. by Zein Ahmed Zein. Gondar, Ethiopia 1986;105-110.
15. Claus D, Berkeley RC. Genus *Bacillus*. In *Bergey's Manual of systematic Bacteriology.* Ed. by John P. Bulter and Victoria Vaughn. Williams & Wilkins, Baltimore, USA. 1986;2:1105-1139.
16. Kovacs N. Identification of *Pseudomonas pyocyaneae* by the oxidative reaction. *Nature* 1956;178:703.
17. Collins CH, Lyne PG. *Microbiological Methods*, 4th ed. Butter worth & Co. std. England 1976.
18. Iigbe IA, Objofeitimi EO, Elegbe I, Akinola MO. Pathogenic Bacterial Isolated From Infant Feeding Teats: Contaminated teats by illiterate and educated nursing mothers in Ile - Lfe, Nigeria. *Am J Dis Child* 1982;136:672-674.
19. Imong SM, Jackson DA, Rungruengthanakit K, Wong Sawadii L, Amatayakul K, Drewett RF, Baum JD. Maternal Behaviour and Socioeconomic Influences on the Bacterial count of Infant weaning Foods in Rural North Thailand *J Trop Pediatr.* 1995;41:234-240.
20. Wolde-Tensay Z, Mengistu A. Indigenous weaning foods: Hygienic and diarrhoeal diseases in rural Ethiopian setting, Jimma Zone. *Ethiop J Hlth Dev.* 1997;11:149-156.
21. Black RE, Brown KH, Becker S, Abdul Alim AR, Merson MM. Contamination of weaning Foods and Transmission of Enterotoxigenic *Escherichia coli* Diarrhoea in children in Rural Bangladesh. *Trans R Soc Trop Med Hyg.* 1982;76:259-264.
22. Imong SM, Rungruengthankit K, Rungyuttikarn C. The Bacterial content of Infant Feeding Foods and water in the Rural Northern Thailand. *J Trop Pediatr.* 1989;35:14-18.
23. Odugbemi T, Oyerinde JPO, Odujinrin OMJ. Bacteriological Study of Cooked Ogi (fermented cereal weaning food) and Its Potential Safety in Rural Nigerian Community. *Trans Roy Soc Trop Med Hyg.* 1993;87:234-235.
24. Ghuliani A, Kaul M. Contamination of Weaning Foods and Transmission of *E.coli* in causation of Infantile Diarrhoea in Low Income Group in Chandigarh. *Indian Pediatr* 1995;32:539-542.

