# BACTERIOLOGICAL ANALYSES OF WEANING FOODS AND GROWTH POTENTIAL OF SOME FOODBORNE PATHOGENS IN WEANING FOODS

BY

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# ABSTRACT

One hundred samples of feeding-bottles were collected from infants and young children coming to four clinics in Addis Ababa, from November, 1996 to April, 1997. Information on sex, age, number of bottles used, educational status of the mother and ingredients of bottle content were collected through interview. The bacteriology of these samples was analysed using standard microbiological techniques.

The analyses showed that all the samples had heavy bacterial contamination, the mean count being in the levei of $10^7$ c.f.u/ml. Only 17% of the samples had count $\leq 10^6$ c.f.u/ml. The two most commonly encountered bottle contents (cow's milk and cereal blend) were the most heavily contaminated. About 52% of the milk and 93% of cereal blend had count more than $10^7$ c.f.u/ml. The contamination level was found to be affected by ingredients of bottles used and educational status of mothers. About 90% of the samples prepared by illiterate mothers had count over $10^7$ c.f.u/ml. The percentages for elementary and high school educated mothers for the corresponding load were 85 and 79, respectively.

Three hundred and sixty-nine bacterial isolates, grouped into 12 genera were identified from the bottle content. Thirty-seven per cent of the samples had 3 isolates, whereas 21% and 16% of the samples were contaminated with 4 and 2 isolates, respectively. The dominant organisms were coliforms (34%) followed by *Staphylococci* (28%), *Bacillus spp.* (19%) *Micrococcus spp.* (14%) and other (3%). Three isolates of *Salmonella spp.* of the same serogroup were also identified. Cow's milk harboured 219(59%) of the isolates followed by gruel made of cereal blend(16%).

A total of 30 factory-produced weaning foods (product A, B and C), and 20 of the two most frequently encountered home-made bottle-contents (cow's milk and cereal blend) were prepared at home by mothers under close supervision. The bacteriological analyses of these foods showed that only 30% of the factory-produced weaning foods had bacterial count over $10^2$ c.f.u/ml(g). The count from the two home-made bottle contents was also low; only 15% of the samples had bacteria $\geq 10^2$ c.f.u/ml. These signifies that food handling and the gap between consumption and preparation must be important in this regard. The organisms isolated from the above 50 samples were *Bacillus spp.*, for they were the only organisms that could tolerate the heat treatment.

The growth potential of *Salmonella sp.* in products 'C' and cereal blend was determined. It was found out that it reached to a level of $10^7$ c.f.u./ml(g) in 12 hours and $10^8$ c.f.u/ml(g) in 16 hours. If weaning foods have initial contamination of $10^3$ c.f.u./ml (g) (which by itself is beyond the threshold level for infants) it reaches to unacceptably high level within 8 hours ($10^6$ c.f.u/ml(g)).

In order to intervene with food borne diseases of infants and children, mothers must be taught of food safety principles. Increasing the number of bottles used to feed infants to three or more could also be helpful in reducing food borne bacterial contamination. More important is teaching and encouraging mothers to consider the use of fermented food products as alternatives, which are cost-effective means of reducing bacterial pathogens in weaning foods.

## 1. INTRODUCTION

Children constitute a large proportion of the world's population , i.e. 31.7%(WHO, 1995 b). Over 44% of the African population are children under the age of fourteen (WHO, 1995 b); and 46.1% of the nearly 53 million Ethiopians are children in the age of fourteen and below (WHO, 1993). However, between 12 to 13 million infants and children of the world die each year before they reach their fifth year, many of them during their first year of life (Motarjemi *et al.*, 1993; WHO, 1995 a).

Based on the data collected in 1990, the World Health Organization estimates that the cumulative risk of dying before the age of 5 years for Ethiopian infants and children is 220 per 1000 live births (WHO, 1995 b). In a one-year follow-up study in the Butajira project, Shamebo *et la.,*(1993) raised the death rate of Ethiopian children under five month of age to 293 per 1000, while infant (0 - 11 months old) mortality rate was 136 per 1000 live births.

Child morbidity is also a major global problem, especially in the developing countries. One of the most common child morbidity in these countries is Diarrhoea. This illness has great potential to expose the sick child to other secondary health complications, and ultimately to death if not properly managed. This happens through various ways: faltering the growth of the child, dehydration , malnutrition, anaemic and making the child susceptible to other bacterial and parasitic infections such as malaria (Bhutta *et al.*, 1996).

Almost all the pathogenic organisms that cause diarrhoea get their way into the gastro intestine of the infants via oral route. Though the sanitary conditions in which the infants are found have

important role in the acquisition of these pathogens (Baltazar *et al.*, 1993), it is by far the weaning foods and the way they are handled and fed that play the significant role.

The objectives of the study were, thus, to determine how exposed infants in Addis Ababa are to contaminated weaning foods, and to what degree can certain weaning foods support the growth of bacterial pathogens. The microbial load of ready-to-consume feeding bottle contents made available to the infant/child and that 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 were determined. Finally the growth potential of *Salmonella* test strains in home-made and manufactured weaning foods were evaluated.

## 2. LITERATURE REVIEW

### 2.1 Infant Mortality and Morbidity due to Diarrhoea

Of the huge infant morbidities, diarrhoeal diseases are the second commonest illnesses next to respiratory infections (Motaijemi *et al*, 1993). While Kumate and Isibasi (1986) estimated the number of annual children's diarrhoeal episode to be 0.75 billion, Motaijemi *et al.* (1993) raised the figure to about 1.5 billion. After analysis of a ten-year publication of reviews and articles, Bern *et al.* (1992 ), however, limited the annual diarrhoeal episodes to 1 billion.

The majority of these incidences occurred in the developing world. Kumate and Isibasi (1986) stressed that in the last twenty-five years, all the important epidemic out breaks have occurred in third world. Further evidences from Guerrant *et* «/.(1986) and Lee *et* «/.(1995) strengthen the above notion. While the former authors put the annual illnesses experienced by a child in the developing world to 3-9, the latter authors claimed that a child in the Sub-Saharan Africa suffers an average of four to five episodes each year. This last rate was in agreement with the finding of Claeson (1988), who reported the same rate in the case of Ethiopian children.

A more comprehensive analysis of the magnitude of the global problem of diarrhoeal diseases (from 1980 up to 1990) was conducted by Bern *et* «/.(1992). According to these authors, veiy high rates occurred among poor cliildren in Latin America, where under-two-year olds experienced 10 or more episodes per year.

The mortality rate of children due to diarrhoeal diseases is veiy high. Tire World Health Organization (1995a) estimates the percentage infant mortality associated with diarrhoea to be 24.7%. Motaijemi *et* «/.(1993), estimated that over 3 million children who had been suffering from

dianhoea died in 1990 alone. This figure matched with the estimate made by Bern *et* «/.(1992), 3.3 million deaths per year. The rate in the Ethiopian children under the age of five is alarming, being in the range of 24 to 62% of all deaths, varying from region to region and from season to season (Claeson.,1988).

## 2.2. The Etiologic Agents of Infantile Diarrhoea

A good number of researches world-wide have been devoted to determine the etiologic agents responsible for diarrhoea of infants and young children. Hulian *et* «/.(1991) undertook a two-year etiological survey of acute diarrhoea in children aged 0 - 35 months in five countries. They studied a total of 3640 cases of diarrhoea and 3279 age- and sex-matched controls, and found out that 68% of the cases and 30% of the controls harboured enteric pathogens. The four most commonly detected pathogens in all the five centres were *Rotavirus* (16% of cases, 2% controls), *Shigella spp.* (11% of the cases, 1% controls), enterotoxigenic *Escherichia coli* (16% of the cases, 5% of controls), and *Campylobacter jejuni* (11% of the cases, 7% of controls). These authors found striking pattern of the prevalence of the first two enteric pathogens among diarrhoeic children: *Rotavirus* was commonest among 6 - 11 month olds, accounting for 20% of all cases in this age group; *Shigella spp.* were commonest among 12 - 23 month old, accounting for 22% and 24 - 35 months accounting for 27% of the cases in the respective age group.

Different strains of *E. coil* associated with infantile dianhoea were also isolated from Ethiopian childeren (Aberra *et al.*, 1995;Sullivan *et* «/., 1994)

Teka *et al.* (1996) identified 11 factors which were significantly associated with dianhoea - related deaths of children in Bangladesh, of which *Shigella jlexineri* infection was one. Added to the list are pathogenic bacteria such as *Aeromonas spp, Salmonella spp., Streptoccus spp., Pseudomonas*

*spp., Yersinia spp., Vibrio Cholera, Staphylococcus aureus, Bacillus cereus, Clostridium spp.* Parasitic protozoa such as *Giardia latnbia, Entamoeba histolitica* and *Cryptosporidium spp.* are also recognized as etiological agents of child diarrhoea (Brain and Cleary, 1991; Candy, 1995; Griffiths and Keuch, 1992; O'Ryan *et al*, 1992; and Sansonetti, 1992). Enteropathogenic virus such as Norwalk-like viruses, enteric *Adenovirus* (Gueirant *et* «/.,1986) and the newly recognized *Picobiranvirus* (Candy, 1995) are part of the list.

## 2.3 Weaning Foods and Risk of Diarrhoea

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 weaning foods, which in many countries are prepared under unhygienic conditions, the risk of getting foodbome dianhoeal pathogens by the infants has increased (WHO, 1989). Brown *et* «/.(1989) and Popkin *et al.* (1990) revealed that the addition of even water, teas, and other non-nutritive liquids to breast-milk doubled or trippled the likelihood of diarrhoea; with supplementation of additional nutritive foods or liquids, the risk of diarrhoea further increased significantly.

From a rural community - based study of diarrhoea in relation to the feeding patterns of infants, Mondal *et al.* (1996) found out that early weaning was associated with an incident rate ratio of 3.02. There is a general agreement between the views and findings of different authorities that incidence of diarrhoea is highest at the second six months of life - the age which coincides with introduction of weaning foods - and declines with age (Bern *et al* ,1992; Brown *et al.*, 1989); Motarjemi *et al.*, 1993)

The most important attribution for increasing dianhoeal incidences with the introduction of supplementary foods is the contamination of the food with enteric pathogens. A study of traditional

weaning foods in Bangladesh showed that 41% of the samples served to weaning-aged children contained *E. call* (Black *et al..*, 1982)- the organism which produces up to 25% of all diarrhoeal episodes of the developmg countries (Motarjemi *et al..*, 1993).

Van Steenbergen *et al.* (1983) reported that out of 214, samples of children's dishes they investigated, 62% contained more than $10^3$ c. f. u. /g Enterobacteriaceae, and 44% of them were unsafe for consumption as they contained more than $10^4$ c. f. u. /g Enterobacteriaceae. Twelve per cent of the samples from the same study contained $10^3$ c. f. u. /g *S. aureus*. After analysing 261 samples from 17 countries, Becker *et* «/.(1994) reported that 50% of infant formulae based on milk and other weaning foods were contaminated with *B. cereus*. A good number of other authors have also dealt with gross contamination of weaning foods by pathogens ( Henry *et al.* , 1990; Iinong *et al*, 1989; Mensali *et al.*, 1990).

There are many reasons for such conatminations. Raw ingredients and water used to prepare food can be the source of contamination. Inadequate cooking, the duration, and conditions of storage are also important in rendering the food unacceptably contaminated. For example, Odugbemi *et al.* (1993) detected fecal coliform contamination up to 2400 c.f.u / ml in 31.3% of Ogi samples (fermented cereal weaning food in Nigeria) despite initial low pH (3.6). In their investigation of bactrial contmination of weaning foods, Henry *et al*. (1990) also noted that multiplication of fecal coliforms occurred when there was a delay of more than four hours between preparation and consumption of food.

Containers and utensils used in the preparation of foods may serve as the source of contamination. In this regard, Elegbe *et* «/.(1982) isolated eight pathogenic bacterial strains from ready-to-be-used infant feeding teats. Like wise Ghuliani and Kaul (1995) isolated *E.Coli* in 72% of the

samples from 100 houses. Zeleke and Hailesellassie (1992) also isolated more than 13 enteric bacterial pathogens from feeding bottles in Addis Ababa.

Bottle-feeding is the most important route in exposure of infants to diarrhoeal pathogens. Jellife and Jellife (1982) emphasized that diarrhoeal diseases due to enteric infections is the main killer of bottle-fed babies. Studies conducted in Ethiopia regarding bottle-feeding showed the same result (Gebresellasie *et al.*,1986). The former authors attributed this to be predominantly due to the difficulty of, or impossibility of keeping liquid foods from becoming contaminated culture medium.

Mohamood *et al.* (1989) compared the relative risks of hospitalized diarrhoea among feeding groups in Basra city, Iraque. They found that exclusively bottle-fed infants aged 2 - 3 months and 4 - 5 months had respectively 54.7 and 36.9 times more chance of getting hospitalized diarrhoea than their counterparts who were exclusively breast-fed. The opinion that breast-feeding protects the infant from various infectious diseases, including diarrhoea, is also supported by the findings of Brown *et al.* (1996).

A number of mechanisms may operate in breast feeding to reduce the frequency of diarrhoea. In the first place, breast-feeding reduces the chance of exposure of the infant to enteric pathogens transmitted by contaminated foods. Secondly, breast milk offers anti infective substances to the infant. Dolan *et al.* (1989) studied inhibition of enteropathogenic bacteria by human-milk whey *in vitro* and observed that 9 strains of *Vibrio cholerae*, 8 strains of *Shigella*, 5 strains of *Salmonella* and 10 enterotoxigenic strains of *E. coli* were inhibited by human-milk whey.

Breast milk can promote intestinal environment and micro flora that inhibits proliferation of enteric pathogens, in addition to enhancing the infants nutritional status, thereby reducing susceptibility to diarrhoea (Popkin, 1990).

## 3. MATERIALS AND METHODS

### 3.1. Ready-to-Consume Feeding Bottle Contents

One hundred samples of bottle content were collected from infants and children who visited four public clinics for treatment. These were Kirios Clinic, Beletshachew Clinic, Higher 18 Clinic and Lidetta Clinic. Each bottle content was shaken thoroughly and 20 ml was transferred into sterile screw-capped test tube. The samples were then taken to the laboratory for bacteriologic analyses within 2 to 3 hours.

The following information was also collected by interviewing the persons who brought the children to the clinics: age and sex of the infants, constituents of the bottle content, number of bottles the infant was fed with during the day, and educational level of the mother or the person who was looking after the infant. The educational level of the mother was put under four categories: 1) illiterate- those who have never been exposed to formal education; 2) elementary level- includes mothers who have been educated up to grade 8; 3) high school level- includes those mothers who received grade 9 to 12 education; 4) collage level- includes those mothers who have been educated at least 2 years beyond secondary school and got a diploma.

### 3.2. 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 (product A, B and C). Product A and C were cereal-based foods mixed with milk, and product B was dehydrated milk from the food industry. The preparation was conducted by experienced mothers at home according to the instructions of the manufactures. About 20 ml of product A and B were transferred into screw-capped test tubes; and about 25 g of product C was

transferred into sterile plastic bag. All of the samples were taken to the laboratory for bacteriological analyses within 2 to 3 hours. Similarly, of the commonly encountered home-made weaning foods, 10 samples were prepared at home, collected aseptically and microbiologically analyzed.

### 3.3. Processing of the Samples

In the laboratory, 10 ml or gram of the samples were taken and homogenized in 90 ml of physiological saline solution, and subsequently diluted. Then 0.1 ml was taken from each dilution and 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 staphycococci, MacConkey agar no. 3 (Oxoid) for isolation and enumeration of coliforms and Xylose Lysine Desoxycholate (XLD) agar (Oxoid) for isolation and enumeration of *Salmonella* and *Shigella*. Except for cultures of MSA, which were incubated for 36 hours, all the rest were incubated for 24 hours at 37°c.

In addition, 2.5 ml of the original samples were transferred into 7.5 ml of tetrathionate broth for the enrichment of *Salmonella*. After 24 hours incubation these cultures were streaked onto XLD agar and incubated for another 24 hours at 37°c. At the end of incubation period, *Salmonella*- and *Shigella*-looking colonies were picked based on their morphology and further characterized using biochemical tests.

After counting aerobic mesophilic bacteria on TGYA using SODECO colony counter (New Burnswick Scientific), ten colonies from each countable plate were picked and transferred into Tryptone soya Broth (Oxoid), and after 24 hours incubation at 37°c, streaked on nutrient agar (Oxoid) for purification purpose. A single colony was then transferred onto slant of Blood Agar

Base (Oxoid), from which inocula were transferred into Tryptone soya Broth for Biochemical tests.

### 3.4. Identification

The following characteristics and tests were used to identity the bacterial isolates into their respective genera.

3.4.1. *Cell Morphology*:

Cell shape, grouping and gram reaction were determined from gram staining of a 24-hour old culture. Potassium hydroxide test was also performed in the determination of gram reaction whenever doubt arose in the gram stain.

The production of endospore was detected by spore staining from a culture grown in nutrient agar composed of peptone, 5.0 g; meat extract, 3.0 g; yeast extract, 1.0 g agar, 15.0 g; distilled water, 100 ml; pH 6.8 as recommended by Claus and Berkeley (1986).

Motility was determined by inoculating a 24-hour culture into sulfur Indole Motility (SIM) medium (Oxoid) and incubating for 24 hours at 37°c.

3.4.2. *Cytochrome Oxidase Test*:

A loopful of the test organism young culture was smeared on filter paper strip soaked in the reagent (which was composed of tetramethyl-phenylenediamine, 1.0 g and ascorbic acid 0.1 g dissolved in 100 ml distilled water). The appearance of dark blue colour within 20 seconds was taken as positive.

3.4.3. *Catalase test*:

This was performed by flooding young cultures with 10% $H_2O_2$ solution. Appearance of effervescence was recorded as catalase positive.

3.4.4. *Indole test*:

The production of indole was tested by putting few drops of Ehrich's reagent into culture grown in tubes of SIM medium. The reagent was composed of p-dimethyl aminobenzaldehyd, 1 g; concentrated HCL, 20 ml; and absolute ethanol, 95 ml observation of pink coloration was taken as indication of positive reaction.

3.4.5. *Test for methyl/Red Reduction and Acetyl Methyl carbinol production*:

These tests were done on cultures grown in Methyl Red Vogous Proskaur broth (Oxoid) incubated at 37°c for 5 days. The broth culture was divided into two. In one of them was put few drops of methyl red solution (0.1 g methyl red dissolved in 300 ml of 95% ethanol and diluted to 500 ml with distilled water). In the other tube was put a knife-point creartin and 4 to 5 ml 40% KOH. Red in the case of methyl red and pink in the case of Voges Proskaur was taken as positive.

For gram-positive endospore-forming bacteria. Vogous Proskaur broth was made according to the recommendation of Claus and Berkeley (1986); proteose peptone, 7 g; glucose, 5 g; sodium chloride 5 g; distilled water 1000 ml; pH 6.5.

3.4.6. *Utilization of Citrate as sole source of carbon*:

Slant of Simmon's citrate medium (Oxoid) was inoculated from a young culture of the test

organism and incubated at 37°c for 24 hours. Appearance of blue color confirmed positive result for the test.

3.4.7. *Production of Hydrogen Sulfide*:

Slant and butt of Triple sugar Iron Agar (Oxoid) was inoculated with young culture of the test organism and incubated at 37°c for 24 hours. Blackenning of the butt was taken as indication of $H_2S$ production. Moreover, the reactions of the butt and slant (whether acidic yellow, or alkaline, red) was considered in the differentiation of gram negative rods.

3.4.8. *Hydrolysis of Urea*:

Slant of urea agar (Oxoid) was inoculated with young culture of the test organism and incubated at 37°c for 24 hours. Appearance of pink colour was indicative of urease production by the organism.

3.4.9. *Oxidation / Fermentation test*:

For Gram negative rods, Hugh and Leifson O/F medium was used as described by Collins and Lyne (1976). The ingredients were peptone, 2 g; glucose, 10 g; sodium chloride, 5 g; dipotassium hydrogen phosphate, 0.3 g; agar, 3 g; distilled water 1000 ml; 0.2% bromo thymol blue, 15 ml, pH 7.1.

For Gram positive cocci, Baired-Parker's modification of Hugh and Leifson medium was used as recommended by Collins and Lyne (1976). The ingredients were tryptone, 10 g; glucose, 10 g' yeast extract, 1 g; agar, 2 g; distilled water, 1000 ml; 0.2% solution of bromocresol purple, 20

ml; pH, 7.2. Acid production (yellow) and growth was sought after incubating the inoculated tubes (both in aerobic an anaerobic condition) at 37°c for 5 days.

3.4.10. *Utilization of Carbohydrates*:

For gram negative bacteria, glucose, lactose, sucrose, mannitol, dulcitol, salicine and xylose were tested. Ten percent filter-sterilized solutions of these carbohydrates were mixed aseptically with pre-sterilized basal medium to make a final concentration of 1%. The tube was stabbed with the test organism and incubated at 37°c for 3-5 days. Acid production, confirmed by appearance of yellow colour was positive for the particular carbohydrate. Baird-Parker's carbohydrate basal medium was used for gram positive cocci. The ingredients were: yeast extract, 1 g; ammonium dihydrogen phosphate, 1 g' potassium chloride, 0.2 g; magnesium sulfate 0.2 g; agar, 12 g; 2% bromo cresol purple, 20 ml; distilled water 1000 ml; pH 7.1.

For Gram positive spore forming rods, the carbohydrate basal medium (Claus and Berkeley 1986) consisted of diammonium hydrogen phosphate, 1 g; potassium chloride, 0.2 g; magnesium sulfate, 0.2 g; yeast extract, 0.2 g; agar, 15 g; distilled water, 1000 ml; 0.04%(w/v) solution of bromocresol purple, 15 ml; pH, 7.0. Filter sterilized carbohydrates were mixed with the above autoclaved medium to make a final concentration of 0.5%, and slanted.

3.4.11. *Hydrolysis of starch*:

One gram of soluble starch was suspended in 10 ml of cold distilled water and mixed with 100 ml of nutrient agar. After autoclaving and pouring into petridishes, the test organisms were streaken on the dried plate. The plates were incubated for 3 to 5 days at 37°c, and flooded with I/KI solution. Clear zone around the colony indicated hydrolysis of starch, while blue-black colour was developed by unused starch.

3.4.12. *Test for DNAse production*:

The test organism from young culture was streaken on DNAse agar (oxoid) and incubated at 37°c for 24 to 36 hours, and flooded with 1N HCl. The appearance of clear zone around the colony, in contrast to white precipitate of the back ground, was recorded as DNAse positive.

3.4.13. *Serological Identification*:

Those isolates which were identified by biochemical tests as *salmonella* spp. were tested serologically by *salmonella* O group antisere A - E and vi (national Bacteriological Laboratory, S - 105 21 Stockholm Sweden).

Identification of cocci was done according to the recommendations of Schleifer (1986). Those cocci which showed predominantly tetrad grouping, catalase positive, and exhibited oxidative utilization of glucose in the O/F test were identified as *Micrococcus* spp. The cluster-forming cocci which were fermentative and catalase positive were considered as staphylococci; and those which produced DNAse were identified as *Staphylococcus aureus*.

The characteristics used to identify gram negative rods are summarized in table 1. Identification of these bacteria was done using the techniques recommended by Baker and Breach (1980),Burnner (1986) and Kersters and De Ley (1986).

The Gram positive rods, which produced endospores under aerobic growth and were catalase positive were considered as *Bacillus* spp. These isolates were identified as *B. cereus* when they exhibited the following biochemical properties: acid form mannitol and xylose negative, vogous proskaur positive, pH in vogous proskaur medium $<6$, citrate utilization as sole source of carbon positive. The other isolates were identified only to the genus level.

Table 1. Tests used for differentiation of gram negative isolates

| Test | *Citrobacter sp.* | *E. coli* | *Enterobacter sp.* | *Klebsiella sp.* | *Proteus sp.* | *Salmonella sp.* | *Alcaligenes sp.* |
|---|---|---|---|---|---|---|---|
| Catalase | + | + | + | + | + | + | + |
| Cytochrome oxidase | - | - | - | - | - | - | + |
| O/F | F | F | F | F | F | F | NC |
| Indole | - | + | - | - | + | - | ND |
| Motility | + | + | + | - | + | + | + |
| Methyl Red | + | + | + | +/(-)* | + | + | ND |
| Voges proskaur | - | - | + | + | - | - | ND |
| Simmon's citrate | + | - | - | +/(-)* | + | + | ND |
| TSI Butt | A | A | A | A | A | A | AL |
| TSI slant | AL | A | A | A | AL | AL | AL |
| TSI $H_2S$ | + | - | - | - | + | + | - |
| Urease | - | - | + | + | + | - | - |
| Lysine decarboxylase | - | + | - | + | - | + | ND |
| Gulucose | + | + | + | + | + | + | - |
| Lactose | + | + | + | + | - | - | - |
| Sucrose | + | + | + | + | +(-)* | - | - |
| Mannitol | + | + | + | + | - | + | ND |
| Dulcitol | + | + | - | +(-)* | - | - | - |
| Salicine | - | + | + | + | - | - | - |
| Xylose | + | + | + | + | + | + | - |

NC = not utilized

* = some strains showed -ve result

### 3.5. Determination of Growth potential of Salmonella test Strain in Selected Foods

One locally manufactured (product C) which consisted of cereals and milk, and one home-made weaning food, commonly encountered-cereal blend consisting of Barley, Oat and Teff in the ratio of 3:2:1, were considered for this test. The two groups of weaning foods were cooked thoroughly and separately transferred into sterile bottles aseptically. They were then inoculated with a *Salmonella* test strain to give an inoculum level of $10^2$ to $10^3$ c.f.u./ml and kept at ambient temperature for 16 h. The *Salmonella* test strain was isolated from the weaning foods considered in this study. Portions of food were taken aseptically at 4-hour intervals from 0 to 16 hours. Appropriate dilutions were surface-plated on Macconkey No.3 agar (Oxoid). The plates were incubated at 37°c for 24 hours and counted. Each test was done in triplicates.

# 4 RESULTS AND DISCUSSIONS

## 4.1 The Study Population

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 6 of the males and 9 of the females were over the age of one year

According to the responses of the mothers to the inquiry about their educational status, 21 were illiterate, 27 had elementary level education, 47 high - school level education and, 5 college level education. Regarding to how many bottles were interchangeably used in a day to feed an infant, 51 infants were fed with the same bottle throughout the day, of which 32 were < 6 months old, 13 were between 7 and 12 months old and 6 were >12 months old.

The ingredients of the feeding-bottles were categorized into six groups: cow's milk, commercial dried 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 such as tea, ***abish,*** rice water, etc. The large proportion of the studied infants and children (60%) consumed cow's milk followed by cereal blend (14%). Of the 48 infants under the age of 7 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 15 infants aged >12 months.

Table 2 shows educational status of the mothers in relation to number of bottles used for feeding in a day and ingredients used for bottle feeding. Over half of the mothers (51%) considered in this study used the same bottle throughout the day in feeding their child. This consisted of 70% of the illiterate and elementary level educated ;mothers, and ≤ 32.69% of mothers educated at

high school or college level. Only 9 of all mothers used three or more bottles, while 40% of the mothers used two bottles. This is in contrast to the finding of Elegbe *et al.* (1982) 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. About 60% of all mothers and over half of the mother's at every level of educational status fed their infants with cow's milk. Except college-level educated mothers, all of whom used only cow's milk as a weaning food, all the other 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. 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 show that less than a quarter of the illiterate mothers used 2 or more bottles. However, more than half of the high school and college level educated mothers used 2 or more bottles in a day to feed their infants.

Table 2. Type of bottle content analysed and number of bottles used in relation to educational level of mothers.

| Educational level mothers | No | Ingredients of bottle content | | | | | | No of bottles used/day | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | 1 | 2 | ≥ 3 |
| Illiterate | 21 | 11 | 1 | 3 | 2 | - | 4 | 16 | 3 | 2 |
| Elementary | 27 | 18 | - | 6 | 1 | - | 2 | 18 | 8 | 1 |
| High School | 47 | 26 | 2 | 5 | 2 | 5 | 7 | 15 | 27 | 5 |
| College | 5 | 5 | - | - | - | - | - | 2 | 2 | 1 |
| Total | 100 | 60 | 3 | 14 | 5 | 5 | 13 | 51 | 40 | 9 |

I= cow's milk; II = dried milk; II cereal blend; IV = milk + cereal; V= cereal + legumes; VI others.

## 4.2. Bacteriological Analyses:-

### 4.2.1. Gross bacterial contamination of feeding bottles

Nearly all of the samples tested had very high gross bacterial contamination. The lowest count observed was $1.6 \times 10^5$ c.f.u/ml, and the highest count was $9.9 \times 10^8$ c.f.u./ml of bottle content. The mean count of the samples was $2.0 \times 10^7$ c.f.u /ml (with standard deviation of $1.1 \times 10^7$ c.f.u. /ml). While only 17% of the bottle contents analysed had bacterial count of $10^6$ c.f.u. /ml and below, 83% showed count of more than $10^6$ c.f.u. /ml. of these, 33% yielded count of more than $1.0 \times 10^8$ c.f.u/ml.

These figures are alarmingly very high when compared to results of other studies conducted else where. Imong *et al.*(1989) reported a mean total bacterial count of $3.8 \times 10^4$ with only 10 per cent recording counts of greater than $10^6$ organisms/g. These authors claimed that the figures they reported were above internationally recommended "safe" levels.

With respect to the relationship between total bacterial count, ingredients of bottle content and educational status of the mothers it is possible to see that the two most commonly consumed bottle contents (cow's milk and cereal gruel) were the most heavily contaminated (Table 3). Only 14 of the 60 samples of cow's milk gave counts between $10^6$ c.f.u. /ml or below; and over half of the cow's milk samples contained $\geq 10^7$ c.f.u/ml. Such high gross bacterial contamination of milk and cereal - based weaning foods was also noted by Black *et al.* (1989), when they investigated etiology of infantile diarrhea in Hasscar, Peru.

Cow's milk was contaminated at different contamination levels with almost a similar frequency. Most of the other bottle food types tended to have contamination at the higher levels ($10^7$ to $10^8$ cfu/ml). In addition to cow's milk (31 of 60), higher level of contamination was also noted for cereal blend (12 of 14). About 90% of the samples from infants of illiterate mothers

contained count of more than $10^6$ c.f.u. /ml. Bottle contents from college level educated mothers had counts between log 5 and log 7 c.f.u./ml. Although this count is less than that observed in the case of mothers with lower level education, it was quite high in the absolute sense. This is in contrary to one's expectation. In fact other studies of weaning foods and feeding bottles showed that the degree of bacterial contamination decreased with increased educational level of the mothers (Elegbe *et al.*, 1989; Imong *et al.*, 1995).

Of the 51 mothers who used single feeding bottle, 32 had bottle content counts of more than $10^7$ c.f.u./ml (Table 4). Similar counts were also noted for mothers who used two feeding bottles (22/40) and three bottles (6/9). Although changing feeding bottles frequently should help to lower the microbial load of the weaning foods, the observation in this study showed the contrary. 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.

Table 3. Total bacterial count in relation with educational level of mothers and bottle- content

| Count in log/ml. | Educational status of mothers | Ingredients of bottle | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | |
| 5.0 - 6.0 | Illiterate | 2 | - | - | - | - | - | 2 |
| | Elementary | 4 | - | - | - | - | - | 4 |
| | High school | 7 | - | - | 1 | 2 | - | 10 |
| | College | 1 | - | - | - | - | - | 1 |
| 6.1 - 7.0 | Illiterate | 3 | - | - | - | - | 1 | 4 |
| | Elementary | 4 | - | - | - | - | 3 | 7 |
| | High school | 4 | - | 1 | - | - | 3 | 8 |
| | Collage | 4 | - | - | - | - | - | 4 |
| 7.1 - 8.0 | Illiterate | 3 | 1 | 1 | 1 | - | 1 | 7 |
| | Elementary | 3 | - | 2 | - | - | - | 5 |
| | High school | 9 | - | 1 | - | 2 | 3 | 15 |
| | College | - | - | - | - | - | - | - |
| Over 8.0 | Illiterate | 3 | - | 3 | 1 | - | 1 | 8 |
| | Elementary | 7 | - | 3 | 1 | - | - | 11 |
| | High school | 6 | 2 | 3 | 1 | 1 | 1 | 14 |
| | Collage | - | - | - | - | - | - | - |
| Total | | 60 | 3 | 14 | 5 | 5 | 13 | 100 |

I= cow's milk; II = dried milk; II cereal blend; IV = milk + cereal; V= cereal + legumes; VI others.

Table 4 Bacterial count in relation to number of bottles.

| No. of Bottles | Bacterial count in c.f.u /ml | | | | Total |
|---|---|---|---|---|---|
| | $10^5$ - $10^6$ | $>10^6$ - $10^7$ | $>10^7$ - $10^8$ | $> 10^8$ | |
| 1 | 7 | 12 | 14 | 18 | 51 |
| 2 | 8 | 10 | 11 | 11 | 40 |
| 3 | 2 | 1 | 2 | 4 | 9 |
| Total | 17 | 23 | 27 | 33 | 100 |

### 4.2.2. Isolation and enumeration of specific organisms

A total of 369 bacterial strains belonging to 12 genera were isolated from the feeding bottle contents considered in this study. The dominant isolates were coliforms (33.3%) followed by staphylococci (29.8%), *Bacillus* spp. (18.9%) and *Micrococcus* spp. (14.1%). 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 by Zeleke and Haileselassie (1992) for weaning foods from Addis Ababa. High coliform incidence in weaning foods were also noted by Motarjami (1993) elsewhere. Total coliform count of weaning foods was reported to be related with food hygiene practices and maternal factors (Imong *et al.*, 1995). The coliforms in this study were dominated by *Klebsiella* spp. (44%). *E. coli* also made up 24% of the coliforms, and this was similar to the 23% reported by Zeleke and Haileselassie (1992), thus indicating similar levels of faecal contamination. This, however, was much lower than the 31.3% reported by Odugbemi *et al.*(1993) for ogi, a fermented cereal weaning food in Nigeria; and the 72.3% reported for samples of weaning foods and other sources of contaminations in India (Ghuliani and Kaul, 1995).

Unlike a previous study on weaning foods in Addis Ababa (Zeleke and Haileselassie, 1992), three *Salmonella* isolates were encountered from bottle contents made of cow's milk and gruel made from cereal blend. All belonged to Group D. These are the two types of weaning foods from which the faecal contamination indicator, *E. coli*, was isolated at a high frequency. 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 and *Salmonella* 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.

Of the 110 isolates of staphylococci, 60.9% were *Staph. aureus*. All types of weaning foods yielded *Staph. aureus* at varying frequencies with cow's milk and cereal blend having the highest frequencies. As *Staph. aureus* is capable of producing heat-stable toxins, all types of weaning foods considered in this study seem to be possible sources of staphylococcal gastroenteritis to the infants. The dominance of *Bacillus cereus* among the *Bacillus* isolates is also of a similar concern as *B. cereus* could cause food poisoning. The majority of *B. cereus* was isolated from cow's milk and cereal blend. Food made of reconstituted dried milk did not yield any *B. cereus* in this study. This was in contrast with the observations of Becker *et al.* (1994) who reported that 54% of dried milk samples were contaminated with *B. cereus*. It is worth noting, however, that *B. cereus* and other *Bacillus* isolates could come into the food as part of the uncooked ingredient as spores could survive cooking temperatures.

## 4.3 Analysis of Weaning foods Prepared under Controlled Conditions.

Only 9 of the 30 factory-produced samples were found to be contaminated with over $10^2$ c.f.u. bacteria /ml or g. The rest had count below $10^2$ c.f.u. /ml or g. The maximum count was 3.3 $x10^5$ c.f.u /ml or g. A total of 9 isolates were detected from the 30 factory-produced weaning foods. All were *Bacillus* spp. The three product types (Product A, B and C) yielded four, three and two isolates, respectively.

Of the 20 home-made weaning foods (cereal blend, and cow's milk) prepared at home by mothers (under close inspection), only 3 samples (two from cereal blend and one from cow's milk) were contaminated with bacteria $\geq 10^2$ c.f.u /ml. Five of them had no bacterial counts, three from cow's milk. The mean count of these samples was 33

c.f.u /ml. All the 3 isolates were *Bacillus* spp. (2 *B. cereus* and 1 other *Bacillus* spp). (Table 6).

The fact that only *Bacillus* species were isolated is a good indicator of the effectiveness of heat treatment of the foods to reduce bacterial contamination. When one compares the contamination level of these foods to those of the bottle contents, one can see 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 re - used utensils, and the feeding bottles themselves. This notion is supported by reports of other workers (Imong *et al.*, 1989; Elegbe *et al.*, 1989). The investigation of Elegbe et al. (1989) 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 (1995) 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.

Table 5. No. of isolates of the organisms identified in feeding bottles in relation with bottle content.

| | No of Isolates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ingredients | Citro-bacter spp | Entero bacter spp | E. coli | Klebs iella spp | Proteus spp. | Salmonella spp | S. aureus | other staphylo cocci | B. cereus | Other Bacillus spp | Micrococeus spp. | Alcaligens spp | Total |
| I | 3 | 19 | 17 | 33 | 3 | 2 | 41 | 26 | 23 | 19 | 32 | 1 | 219 |
| II | - | 1 | 1 | 1 | - | - | 2 | 2 | - | 2 | 2 | - | 11 |
| III | 2 | 4 | 7 | 10 | 3 | 1 | 9 | 6 | 6 | 5 | 7 | - | 60 |
| IV | 1 | 1 | 2 | 2 | - | - | 2 | - | - | 2 | 1 | - | 11 |
| V | - | 2 | 1 | 3 | - | - | 3 | 2 | 4 | 3 | 4 | 1 | 23 |
| VI | 2 | 5 | 2 | 6 | - | - | 10 | 7 | 5 | 1 | 6 | 1 | 45 |
| Total | 8 | 32 | 30 | 55 | 6 | 3 | 67 | 43 | 38 | 32 | 52 | 3 | 369 |

I= cow's milk; II = dried milk; II cereal blend; IV = milk + cereal; V= cereal + legumes; VI others.

Table 6. Total mean count, and number of isolated of weaning foods prepared at home.

| | | | | No. of isolates | | |
|---|---|---|---|---|---|---|
| Source of sample | Food | No. of samples tested | mean total count in log/ml | Bacillus cereus | Other Bacillus spp | Total |
| Industrial | A | 10 | 1.794 | 2 | 1 | 3 |
| | B | 10 | 2.169 | 1 | 1 | 2 |
| | C | 10 | 2.650 | 1 | 3 | 4 |
| Total | | 30 | | 4 | 5 | 9 |
| Home-made | I | 10 | 1.780 | 1 | 1 | 2 |
| | II | 10 | 1.262 | 1 | - | 1 |
| Total | | 20 | | 2 | 1 | 3 |

I= cow's milk, II=cereal blend

## 4.4 Growth Potential of *Salmonella* spp. in Weaning Foods.

The potential of *Salmonella* spp. to grow in weaning foods was determined in one of the frequently used factory-produced foods (product 'C') and one of the home - made food (gruel made from cereal blend). In both of the food items tested, the organism grew at fast rate and increased by about 4 log units within 8 hours and reached levels as high as log 8 c.f.u./ml within 12 hours. It reached to the level of $2.76 \times 10^8$ c.f.u /ml in gruel of cereal blend, and $5.1 \times 10^8$ c.f.u/g in product 'C' within 16 hours. In most low-income households, weaning foods are usually cooked early in the day and kept in containers at ambient temperatures. Feeding bottles are filled from these containers. Early contamination with *Salmonella* could, thus, result in its multiplication either within the storage container or within the feeding bottles.

*Salmonella* was reported to reach the same level of growth in fermenting "ergo" in overnight growth (Mogessie, 1993). But the growth in "shiro wot" reported by Mogessie (1996) was slightly higher than that obtained in this study. Its growth in the weaning foods was higher than the one reported by Mezgebu (un published ) in "kitfo" for equal incubation time (12 hours). However, the number reached by *Salmonella* as observed by the various authors was high enough to cause gastroenteritis even by those strains which require to reach high doses to cause food infection.

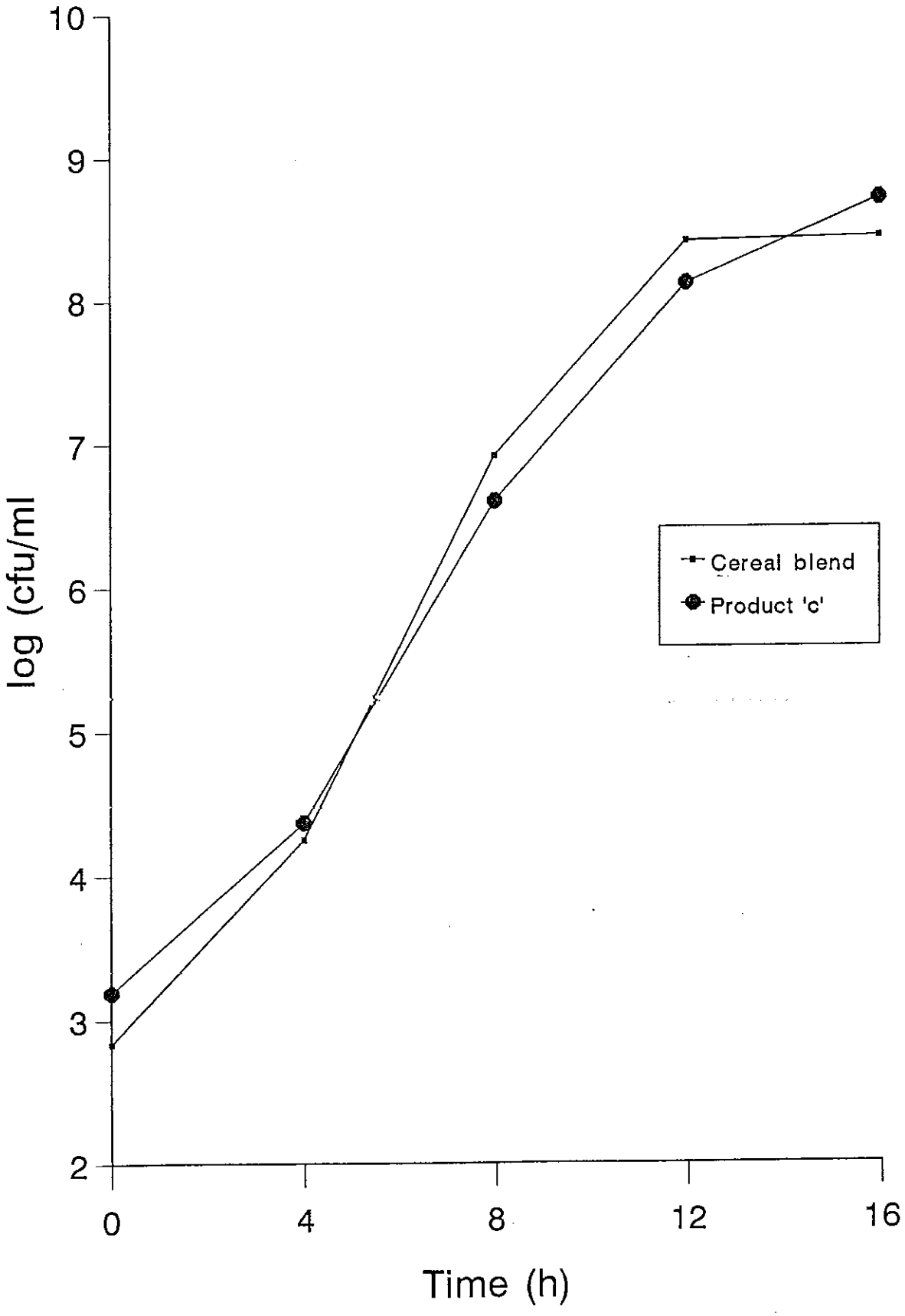


Fig. 1. Growth dynamics of *a Salmonella* test strain in two weaning foods

# CONCLUSIONS AND RECOMMENDATIONS

This project has generated important information about the bacteriological status of weaning foods in Addis Ababa. First, both educational level of mothers and ingredients of bottle content are important factors in determining the level of bacterial contamination. Secondly, it would be fair to infer that most of the infants in this city are fed with feeding bottles which are heavily contaminated with bacteria. More important is the fact that the level of pathogenic bacterial is high enough to put the infants at potential danger. According to Snyder. (1986), *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* should be below 5, $10^3$, $10^2$, and $10^3$ respectively for a baby to tolerate them. That most of the contaminants of feeding bottles were faecal coliforms shows that faecal contamination is the major source of this problem.

Since it is the food handlers who are responsible for the contaminations of weaning foods, effective intervention against food borne diseases of infants should focus on educating mothers about food safety principles. This programme should be designed taking into consideration the hazards associated with feeding - bottles, and the socioeconomic condition of the society.

As the number of bottles used affects the contamination level, usage of three or more bottles; and effective cleaning method such as boiling in water for 10 minutes, should be taught to mothers.

Another important means to reduce food borne disease of infants is to use fermented foods for weaning infants. These foods have been found to be very effective in precluding pathogenic

bacterial from reaching unacceptable level of contamination as the pH within them is too low for the growth of pathogens, and as some of the lactic acid bacterial produce bacteriocine (Nout *et al.*, 1989; Odugbemi *et al.*, 1994; Lewus *et al.*, 1991; Ayele and Berhanu, 1994; Yusof *et al.*, 1995).

# REFERENCES

**Aberra Geyid, Olvisk, O., and Ljungh, A. (1995):** Virulence Properties of *Escherichaia coli* Isolated from Ethiopian patients with Acute or Persistent Diarrhoea. A Ph.D. dissertation. I:1-I:13

**Ayele Nigatu and Berhanu Abegaz (1994):** Inhibition of spoilage and Foodborne Pathogens by Lactic Acid Bacteria Isolated from Fermenting *tef* (*Eragostis tef*). *Ethiop. Med. J.*, 32 (4) 223-229.

**Baker, F.J., and Breach, M.R. (1980):** *Medical Microbiology Techniques.* Butter worth & Co. (Publishers) ltd., London - Boston.

**Baltzar, J.C., Tigola, T.V., and Temponko, S.B. (1993):** Hygiene Behavior and Hospitalised severe childhood Diarrhoea: a case - control study. *Bull WHO.*, 71: 323 - 328.

**Becker, H., Schaller, Von - Weise, W., and Terplan, 6.(1994):** *Bacillus cereus* in Infant Foods and Dried Milk Products. *Int. J. Food Microbiol.*, 23:1-15

Bern, C., Martines, de Zoysa, I., and Glass (1992): The magnitude of Global Problem of Diarrhoeal Diseases: a ten-year update. *Bull WHO*, 70:705 - 714

**Bhutta, Z.A., Punjwani, N., and Lindblad, B.S.(1996):** Concomitant Bacteremea As a Risk factor for Diarrhoeal Disease Mortality in Karachi: a case control study of hospitalized children. *Acta Paediatr.*, 85:809 - 813.

**Black, R.E., Brown, K.H. Becker, S., Abdul Alim, A.R., and Merson M.M. (1982):** contamination of weaning Foods and Transmission of Enterotoxigenic *Escherichia coli* Diarrhoea in children in Rural Bangladesh. *Trans. R. Soc. Trop. Med. Hyg.*, 76:259 - 264.

**Brain, M.J., and Clearly, T.G. (1991):** Colonic Infections. *Cur. Opin. Gastrointerol.*,
11:76-82.

**Brown, K.H, Black, R.E., de Romano. G.L., and de Kanashiro, H.C. (1989):** Infant-feeding Practieces and thier Relation ship with Diarrhoeal and other Diseases in Hauscar (Lima), Peru. *Pediatrics*, 83:31-40

**Burnner, D.J. (1986):** Enterobacteriace. In *Bergey's Manual of systematic Bacteriology*. Ed. by Noel R. Krieg and John G.Holt Williams & Wlikins, Baltimore, USA. Vol:1 408- 420

**Candy, D.C.A. (1995):** Gastrointestinal Infections in Children. *Cur. Opin. Gastrointerol.*, 11:76-82

**Claus, D., and Berkeley, R.C., (1986):** Genus *Bacillus*. In *Bergey's Manual of systematic Bacteriology*. Ed. by John P.Bulter and Victoria Vaughn. Williams & Wilkins, Baltimore, USA. Vol. **2** :1105 - 1139

**Cleason, M. (1988):** Diarrhoeal diseases. In *The Ecology of Health and Diseases in Ethiopia*. Ed. by Zein Ahmed Zein. Ministry of Health, Addis Ababa, Ethiopia. **244** - 251

**Collins, C.H. and Lyne, P.G. (1976):** *Microbiological Methods*, 4th ed. Butter worth & Co. std. England.

**Dolan, S.A., Boesman - Finkelstein, M., Finkelstein, R.A. (1989).** Inhibition of Entero- pathogenic Bacteria by Human Milk whey *in vitro*. Peditr. Infect. Des J., **8** : 430 - 436.

**Elegbe, I.A., Objofeitimi, E.O., Elegbe, I., and Akinola, M.O. (1982):** Pathogenic Bacterial Isolated From Infant Feeding Teats: Contaminated teats by illitrate and educated nursing mothers in Ile - Lfe, Nigeria. *Am.J.Dis. Child.* **136**:672 - 674

**Gebreselassie Okbagzhi, Etsegenet Gedlu, Eyasu G. Medhin, Eyob Tsegaye, Fatuma Abki, and Mahteme Baye (1986):** Infant Feeding Practices in Debre Tabor. In *Gondar College of Medical Sciences IX*th *Public Health and Research Conference*. Ed. by Zein Ahmed Zein. Gondar,

Ethiopia 105 - 110.

**Ghuliani, A., and Kaul, M. (1995):** Contamination of Weaning Foods and Transmission of *E.coli* in causation of Infantile Diarrhoea in Low Income Group in Chandigarh. *Indian Pediatr* **32**:539 - 542.

**Griffiths, J.K., and Keusch, G.T. (1992):** Colonic Infections. *Cur. Opin. Gastroenterol.*,**8**. 87- 97.

**Guerrant, R.L., Lohr, J.A., and Williams, E.K. (1996):** Acute Infectious Diarrhoea : I. Epidemiology, Etiology and Pathogenesis. *Pediatr. Infect. Dis.*, **5**:353 - 359.

**Henry, F.J., Patwary, Y., Huttly, S.R., and Aziz, K.M. (1990):** Bacterial contamination of Weaning Foods and Drinking water in Rural Bangladesh. *Epidemiol Infect.*, **104**:79- 85.

**Hulian, S., Zhen, L.G., Mathon, M.M., Mathew, M.M., Olarte, R.E., Mang, U.K., ghafor, M.A., Khan, M.A., Sami, Z., and Sutton, R.G. (1991):** Etiology of Acute Diarrhoea Among Children in Developing Countries: a multicentre study in five countries. *Bull WHO.*, **69**:549 - 555.

**Imong, S.M. Jackson, D.A, Rungruengthanakit, K., Wong Sawadii, L., Amatayakul, K., Drewett, R.F., Baum, J.D. (1995):** Maternal

Behaviour and Socioeconomic Influences on the Bacterial count of Infant weaning Foods in Rural North Thailand. *J. Trop. Pediatr.*, **41**: 234 - 240.

**Imong, S.M., Rungruengthankit, K., and Rungyuttikarn, C. (1989):** The Bacterial content of Infant Feeding Foods and water in the Rural Northern Thailand. *J. Trop. Pediatr.*, **35**: 14 - 18

**Jelliffe, D.B., and Jelliffe, E.F. (1982):** Feeding Utensils and Infant Diarrhoea in Less Developed countries . *Am. J. Dis. Child,* **136**:667

**Kesters, K., and De Ley, J. (1986):** Genus *Alcaligenes* Castellani and Chalmers 1919, 936 [AL]. In *Bergey's Manual of Systematic Bacteriology*. Ed. by Noel R. Krieg and John G. Holt. Williams & Wilkins, Baltimore, U S A Vol. **1**: 361-373.

**Kumate, J., and Isibasi, A. (1986):** Pediatric Diarrhoeal Diseases: a global prospective. Thrasher International conference on Pediatric Enteric infection. *Pediatr. Infect. Dis.*, **5**: 521 - 528

**Lee, L.A., Dogore, R., Redd, S.C., Dogore, E., Metcock, B., Diabate, J., van Assendelf, O.w., Deccock, K., Patrick, E., and Herrington, J. (1995):** severe Illness in African children with Diarrhoea: implications for case management strategies. *Bull WHO.*,73: 779-785

**Lewus, C.B., Kaiser, A., and Montville, T.J. (1991):** Inhibition of

Foodborne Bacterial Pathogens by Bacteriocins from Lactic Acid Bacterial Isolated from Meat. *Appl. Environ. Microbiol.*, 57:1683 - 1688.

**Mensah, P.P., Tomkins, A.M., Drasar, B.S., and Harison, T.J. (1990):** Fermentation of cereal for Reduction of Bacterial contamination of weaning Foods inGhana. *Lancet*, **336** (8708):140 - 143

**Mezgebu Tegegn (1997, unpublished):** Prevalence and Growth potential of *Salmonella* in "kitfo" M.Sc. Thesis Dissertation, Addis Ababa University.

**Mogessie Ashenafi (1993):** Fate of *S. enteritidis* and *S. typhimurium* during the fermentation of "ergo" a traditional Ethiopian sour milk. *Ethop. Med.J.*, 3(12): 91-98

**Mogessie Ashenafi (1996):** Growth Potential of some Foodborne Pathogens in Various Traditional Ethiopian Savces. *Ethiop. J. Hlth. Devt.*, **10**(1): 41-45

**Mahamood, D.A., Feachem, R.G., and Huffly, S.R.A. (1989):** Infant Feeding and Risk of Severe Diarrhoea in Basra City, Iraq : a case - control study. *Bull WHO.*, 67:701 706

**Motarjemi, Y., Kaferstein, F., Moy,G., and Quevedo, F. (1993):** Contaminated Weaning Food: a major risk factor for diarrhoea and

associated malnutrition. Reviews / Analyses. *Bull WHO.*, **71**: 79 - 92

**Mondal, S.K., Sen Gupta, P.G., Gupta, D.N., Gosh, S., Sikde, S.N., Rajendran,K., Saha, M.R., Sircar, B.K., and Bhattachrya, S.K. (1996):** Occurrence of Darrhoeal Diseases in Relation to Infant Feeding Practieces in a Rural Community in West Bengal, India. *Acta Paeditr.*, **85**:1159 - 1162

**Nout, M.J.R., Rombouts, F.M., and Have laar, A. (1989):** Effect of Accelerated Natural Lactic Fermention of Infant Food Ingredients on some Pathogenic Microorhanisms. *Int. J. Food Microbiol.*, **8**:*351 - 361*

**Odugbemi, T., Oyerinde, J.P.O. and Odujinrin,O. M.J. (1993):** Bacteriological Study of Cooked Ogi (fermented cereal weaning food) and Its Potential Safety in Rural Nigerian Community. *Trans. R. soc. Trop. Med. Hyg.*, **87**: 234 - 235.

**O'Ryan, M.L. Maston, D.O., and Pickering, L.K., (1992)** Pediatric Gastroenteritis. *Cur. Opin.Infect.Dis.*, **5**:99- 105

**Popkin, B.M., Adiar, L., Akin, J.s., Black, r., Briscoe, J., and Flieger, W. (1996):** Breast - feeding and Diarrhoeal Morbidety. *Pediatr.*, **86**:874 - 882

Sansonetti, P.J.(1992) : *E.coli, Shigella,* Antibiotic Associated Diarrhoea and Prevention and Treatmetn of Gastroenteritis *Cur. Opin. Infect. Dis.*, 5: 66 - 73.

**Schleifer, K.H. (1986)** : Family Micrococcace. In *Bergey's Manual of stystematic Bacteriology.* Ed. by John P. Butler and Victoria M. Vaugh. Williams & Wilkins, Batlimore, USA.

**Shamebo, D., Sandstorm, A., Muhe., L., Freijic,. L., Krantz, I., Lonnberg, G., and wall, S. (1993):** The Butajira Project in Ethiopia: a nested case - reference stydy of under - five mortality and its public health determinants. *Bull WHO.*, **71**:389

**Snyder, O.P.Jr. (1986):** applying the Hazard Analyses Critical control system in Food Service Foodborn Illness Prevention. In World *Congress Foodborne Infections and Intoxications* Vol II, 1018 - 1024.

**Sullivan, P.B., Coles, M.A.T., Aberra Geyid, and Ljungh, A. (1994):** Enteropathogenic and Enteroadherent - aggregative *Escherichia coli* in children with Persistent Dearrhoea and Malnutrition. *Ann. Trop. Pediatr.*, **14**:105 - 110

**Teka, AS.G., Farugue, ASG., and Fuchs, G.J. (1996):** Risk Factors for Deaths in Under - age-five Children Attending a Diarrhoea Treatment Centre. *Acta Pediatr.*, **85**:1070 - 1075.

**Van Steenbergen, W.M., Mossel, D.A.A., Kasin, J.A., and Jaiven, A.A.J. (1983):** Machackos Project studies: agents affecting health of mother and child in a rural area of Kenya. *Trop. Geo. Med.*, **35**: 193 - 197

**World Health Organization - (1989):** Research On Improved Infant Feeding Practices to Prevent Dearrhoea or Reduce its Severity: Memorandom from a JHU/WHO Meeting. *Bull WHO.*, **67**:27 - 33.

**World Health Organization (1993):** Vital Statistics and Life Tables, Table c-1.*Wld. Hlth. Statist Ann.* 1992. C-3.

**World Health Organization (1995a):** Integrated Management of the Sick Child. *Bull WHO.*, **73**:735 - 740.

**World Health Organization (1995b):** Demographic Data. *Wld. Hlth Statist. Ann, 1994.* A.3 - A.8.

**Yusof ,R.M., Baker ,T.A. ,Morgan ,J.B. ,and Adams ,M.R. (1995):**Effect of Ragi and L-lactate-producing Cultures on Enteric Pathogens in a Rice-based Weaning Food. .*Wld .J.Microbiol. Biotechnol. ,11 (6) : 654-657.*

**Zeleke W. Tensay and Hailesellassie Tesfaye (1992):** Isolation of Enteric Pathogens and Coliform Bacteria From Infant Feeding Bottle Content in Addis Ababa, Ethiopia. *Ethiop.J.Hlth Devpt.*, **6**:1-4.