SURVEY OF GASTRO ENTERITIS CAUSING BACTERIAL PATHOGENS IN FEEDING BOTTLE CONTENTS IN ADDIS ABABA

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ABSTRACT

Gastroenteritis causing bacterial pathogens were studied in infant feeding bottle-contents, and stool samples collected from 244 bottle fed babies who were brought to clinics and hospitals from January 1989 to November 1989, in Addis Ababa, Ethiopia. The most frequent bacterial isolates were coliforms which included Enterobacter spp. Klebsiella spp, fecal E. coli, and Citrobacter spp. Enteric Pathogens, like enteropathogenic E. coli, Shigella spp, Staph. aureus, and Bacillus cereus were isolated respectively from 9(3.7%), 1(0.004%), 9(3.7%), and 1(0.004%) of the 244 samples of bottle-contents. The bacterial isolates from feeding teats were similar to those isolates from bottle-contents. Enteropathogenic E. Coli was the predominant pathogen isolated from stool samples of the babies. It was isolated in greater frequency from stool samples of babies with gastroenteritis than from those without gastrointestinal illness. Most of the enteropathogens tested were resistant to commonly available antibacterial agents such as penicillin, tetracycline and ampicillin, and multiple drug resistance was very common among the isolates.

The predominance of coliforms, and the high bacterial counts in the bottle-contents reflect an objectionable bacteriological quality of infant food.

The isolation of enteric pathogens both from bottle-contents and stool samples of the bottle-fed babies may be an indication of the role played by bottle feeding in the transmission of these pathogens in the studied population. Neither the difference in the methods of handling care of bottles used by the nursing mothers, nor the level of mothers' education seems to show a significant difference in the bacteriological quality of the bottle-contents in terms of the total bacterial counts per ml. of samples.

Consistent emphasis on the superior quality of breast milk, and continual teaching of bottle- feeding mothers on better handling of feeding bottles and feeds, accompanied with regular home visit by health workers to observe hygienic condition of house environment are recommended if we are to reduce gastroenteritis in babies as a result of using feeding bottles.

INTRODUCTION

Despite advances in the treatment of acute diarrhoeal disease in recent years, gastroenteritis persists as the major cause of morbidity in infancy and early childhood in many areas of the developing world (Gordon, 1971; Mutanda, 1980; Mata, 1986). In these developing nations as high as six to twelve episodes of diarrhoea per child per year may occur (Mata, 1986), and the episodes are generally associated with infectious diseases such as measles, and pneumonia (Pikering, 1985). According to Snyder and Merson (1982), the estimated total yearly morbidity and mortality from diarrhoeal disease for children under five years of age in Africa, Asia (excluding China), and Latin America, were 744-1000 million episodes and 4.6 million deaths.

Studies of diarrhoeal etiology (WHO, 1978; Mata, 1986; Black et al., 1989) have associated diarrhoeal episodes with various micro-organisms. These micro-organisms appear to be transmitted to infants in the home through contaminated food, water, animal feces and direct person -to- person contact (Black et al., 1989). Among the micro-organisms important in gastroenteritis, bacteria have been implicated in about 20-30 per cent of diarrhoeal cases (Ganyal et al., 1977). The most frequently encountered bacterial pathogens are Salmonella, Shigella, enteropathogenic Escherichia coli,(EPEC) enterotoxigenic E.coli,(ETEC) enteroinvasive E. coli(EIEC),enterotoxin producing Staphylococcus aureus, Vibrio spp; Bacillus cereus, and Clostridium perfringens (WHO, 1978). In addition to these

classical pathogens, a large number of other enteropathogens have been recently recognized as causative agents of diarrhoea. These include a new group of diarrhoeagenic E.coli, Campylobacter jejuni, and Yersinia enterocolitica (WHO, 1980; Matusaki et al., 1984; Levine, 1987). Other bacterial enteropathogens, viruses such as rotavirus, protozoa, helminthes, and fungi have been also implicated in diarrhoeal etiology (Frej, 1973; WHO, 1978, Black et al., 1980; Guerrant et al ., 1983; Zaki et al ., 1986).

Since the standards of personal hygiene and public sanitation is low in many communities of developing countries, contamination of infant feeds with pathogenic micro-organisms may be an important source of infectious diarrhoea (Black et al., 1989). Bacteriological investigations of infant feeds and feeding utensils from Africa and elsewhere (Cherian et al., 1985; Andersonet al., 1970) have demonstrated gross contamination of feeding utensils and feeds.

In Ethiopia, gastroenteritis has been a major disease problem among infants and young children (Freij, 1973; Stintzing et al., 1977; Thoren et al ., 1982). A more recent and comprehensive report (WHO, 1987) indicates that diarrhoea continues to be a major cause of morbidity in Ethiopia. The above report indicates an estimated 39,000,000 episodes per year and approximately 230,000 deaths annually in children under five years of age. Although diarrhoeal disease is the leading health problem in infancy and early childhood, bacteriological studies of infant

feeds and feeding utensils, and its influence on the health of bottle-fed babies in Ethiopia are very scarce. Therefore the aim of the present work is to investigate the importance of infant feeding bottle-contents as a vehicle in transmission of enteric bacterial pathogens in Addis Ababa. The specific objectives of this study include :

1) To determine the total bacterial flora of infant feeding bottle-contents,
2) To isolate and identify bacterial pathogens from the contents of feeding bottles,
3) To isolate possible enteric pathogens from the stools of the bottle-fed babies and correlate with the pathogens from the bottle-contents and teats.

The present study does not attempt to isolate intestinal pathogens which have special isolation requirements such as Campylobacter jejuni, Yersinia enterocolitica or enterotoxigenic Escherichia coli. It mainly focuses on detecting the classical bacterial pathogens such as enteropathogenic E. coli, Shigella, Salmonella, Staphylococcus aureus and other bacteria that can be isolated by routine bacteriological techniques.

LITERATURE REVIEW

1. Infant Feeding Practice and Risk of Diarrhoea

Some what more than a century ago, the care and particularly, the feeding of infants led to the development of pediatrics as a medical speciality (Cone cited in Barness, 1987). Results of many investigations (Cunningham, 1978; Kovar et al., 1984; Chen and Li, 1988) have indicated an association between infant feeding practice and infant health. The majority of these studies demonstrate that infant morbidity and mortality are influenced by the mode of infant feeding practice. According to Machale et al. (cited in Jason et al., 1984), infant mortality is one of the characteristics that distinguish populations in less developed countries from most developed countries. Jason et. al. (1984) have shown that infant mortality is up to ten times higher in many less developed countries than in the United States and Northern Europe. This reflects the difference in sanitation, nutrition, housing and other indicators of socio-economic status (French, 1967; Agarwal et al ., 1982; Jason et al., 1984).

1.1. Breast Feeding

Breast feeding is one kind of biological nutrition. Biological nutrition is arranged before birth via blood of the mother and after birth by the mother's milk (Taba, 1970). Human milk was the only successful infant food until the advent of scientific pediatrics; the invention of electric refrigeration

and the development of formula milk (Barness, 1987). Most infants who are exclusively breast-fed show normal growth until 4 to 6 months of age, although after that time an increasing proportion of infants require supplementation in order to maintain growth (Taba 1970; WHO, 1978).

Mothers milk is superior to artifical feeds at least for the first few months of age. First of all, it is free of the potential hazards associated with artificial feeding which includes the risk of contamination of feeding bottles, rubber teats, and milk which so often results in acute diarrhoeal disease (WHO, 1978, Black et al ., 1989; WHO, 1989;Brown et al., 1989). Most studies (Plank and Milanesi, 1973; Almorth et al., 1982, Kovar et al ., 1984) have demonstrated an association between breast feeding and decreased rate of gastroenteritis. Moreover, a number of these studies have shown that mothers milk has intrinsic anti-infective properties and possibly improved nutritional status during early infancy.

1.2. Bottle Feeding

Although breast feeding was recognized as the best infant feeding practice as early as Biblical times, women have sought alternative methods because of work demands societal need or health requirements (Barness, 1987). A major stimulus to the development of artificial feeds arose in the mid-18th century(Barness, 1987). Today bottle feeding has become a common practice in developed countries and in urban communities of the

developing world. The increasing incidence of bottle feeding of infants in Africa reflects the absorption of the Western ways of life (Drejer, 1980). Artifical feeding of infants is a method which for success relies upon the maintenance of high degree of hygiene in the home. Inadequately cleaned and sterilized feeding equipment increase the risk of transmitting gastrointestinal illness and other infections (Beila et al., 1985).

In developed countries with good sanitation, nutrition and medical care, bottle feeding is less risky than in the set up of the developing countries (Drejer, 1980; Kovar et al., 1984). The substantial risk of bottle feeding to an infant's health and even his/her survival has been well documented (Philip et al., 1969; Surjono et al., 1980; Jelliffe and Jelliffe, 1982). A study in rural Chile (Plank and Milanesi, 1973) showed that the bottle-fed baby in the developing world, all other things being equal, has death rate of 2.3 times his breast-fed peers. Another study (David and David, 1984) indicated that the bottle-fed infants under 6 months of age had a markedly increased risk of severe malnutrition over those fully breast fed. According to Jelliffe and Jelliffe (1982) the danger of bottle feeding stems from the difficulty of keeping liquid feeds and feeding utensils from becoming contaminated by pathogenic microorganisms. Surjono et al. (1980) explain that in addition to marasmus (protein calorie malnutrition) from over dilution of expensive formula milk, diarrhoeal disease, principally due to enteric pathogens, is the killer of bottle-fed babies.

Barrel *et al* . (1980) studied the bacteriology of weaning foods in a rural West African environment, and found a gross bacterial contamination of both commercial milk products and indigenous weaning foods. The production of a sterile feed for a baby depends on a number of factors, the most important of which are, effectiveness of utensil sterilization, the degree of contamination of the original feed materials, and the measures taken to sterilize these feed materials, and personal hygiene of the food handlers (Philips *et al*., 1969; Barrel *et al*; 1979; Barrel *et al* ., 1980). Hence the increased rate of diarrhoea that is observed with introduction of weaning foods indicates that these foods are important vehicles for the transmission of enteric pathogens.

2. Some Etiologic Agents In Gastroenteritis

2.1. Enteropathogenic *Escherichia coli* (EPEC)

Escherichia coli is the only member of the genus *Escherichia* and sub-division of the species is done by means of serological typing (Cooke, 1985). The genus *Escherchia* is composed of motile or non-motile bacteria that conform to the definition of *Enterobacteriaceae* (Edward and Ewing, 1972). Biochemical reactions of *E. coli* compared with other *Enterobacteriaceae* are given in Edward and Ewing (1972).

E. coli is the predominant bacterial species among the facultative normal intestinal flora of warm-blooded animals

(Cheesbrough, 1984; Cooke, 1985). Nevertheless, the presence of E. coli strains capable of causing diarrhoea was recognized as early as 1885 by Escherich (cited in Gross, 1983). According to Levine (1987) there are five catagories of diarrhoeagenic E. coli.

These are :

1) enteropathogenic E. coli (EPEC)
2) enterotoxigenic E. coli (ETEC)
3) enteroinvasive E. coli (EIEC)
4) enterohemorrhagic E. coli (EHEC)
5) enteroadherent E. coli (EAEC)

Enteropathogenic E. coli (EPEC) is one of the classical enteropathogens (WHO, 1978). Epidemiological studies that included serotyping incriminated strains of EPEC to be responsble for outbreaks of infantile diarrhoea in Britain during the 1940s (Bray, 1945 cited in Law, 1988). Since then studies in many locations showed that EPEC can produce diarrhoeal disease. Earlier epidemiological studies in Europe and North America indicated that there were many serious EPEC enteritis up to the 1970's but today EPEC enteritis appears to be of minor importance in these areas due to high standard of hygiene (Gross, 1985). On the other hand, EPEC are still a common cause of enteritis in tropical countries and among communities with poor standard of hygiene (Gross, 1985; Lubwama, 1986; Coiro et al., 1987). Robins - Browne et al. (1980) investigated the etiology of

summer diarrhoea in black South African infants, and the results showed that EPEC were responsible for 43 per cent of the diarrhoeal cases. Househam et al. (1987) studied enteropathogens associated with acute infantile diarrhoea in the black south African community, and reports that EPEC were the second most frequent enteropathogen isolated. Araya et al. (1985) surveyed diarrhoeal disease in children under seven years of age in peri-urban area of Chile and the results of their study show that EPEC was at the top in the list of the total bacterial isolates. A similar study in Nigeria (Akinterinwa et al., 1982) demonstrated that EPEC was the most frequent bacterial pathogen from the study group. This result was confirmed by several other workers (Thoren et al ., 1982; Coiro et al ., 1987; Antai et al ., 1987).

Epidemiological studies in the 1940's in Britain showed that contaminated feeding utensils, milk feeds, and poor hygiene were important vehicles for outbreaks of infantile EPEC enteritis (Gross,1983). Recent microbiological studies of infant feeds and feeding utensils (Elegbe et al ., 1982, Cherian et al ; 1985) have shown that E. coli is the predominant bacterial isolate. Elegbe et al . (1982) studied the bacteriology of infant feeding teats in Ile Ife, Nigeria, and found out that 48 per cent of all isolates were E. coli and out of these, 24.3 per cent were serologically proved to be EPEC strains. Cherian et al. (1985)

recovered several pathogens from infant feeding bottles and teats among which *E. coli* was the most frequently occurring isolate.

In both developed, and developing countries, the etiologic significance of isolating of EPEC from sporadic cases of infantile enteritis has been vague and controversial. This is because EPEC strains can be isolated from patients as well as from age - matched controls (Cooke, 1974; Goldwater *et al.*, 1981). Nevertheless, studies in many places indicated that EPEC strains were isolated with greater fequency from infants with diarrhoea than from healthy infants (Edelman *et al.*, 1983; Lubwama, 1986; Moyenuddin *et al.*, 1987; Law, 1988).

Although the ability of EPEC strains to cause dirrhoea has been confirmed, the mechanism by which they induce a fluid response in human gut has not been fully understood (Gross, 1985; Law, 1988). Recent investigations suggest certain factors that may be of importance in the pathogensis of EPEC diarrhoea. These include the production of Shiga- like toxin, and intestinal adhesion (Gross, 1985; Law, 1988).

2.2. *Shigella*

The genus *Shigella* is composed of non-motile bacteria that conform to the definition of the family *Enterobacteriaceae* and the tribe *Escherichiae* (Edward and Ewing, 1972). The genus comprises four sub-groups, *Shigella dysenteriae*, *Sh. flexneri*,

Sh. boydii, and Sh. sonnei. The first three sub-groups may be further sub-divided by serotyping. There are 10 serotypes of Sh. dysenteriae, 8 of Sh. flexneri, and 15 of Sh. boydii (WHO, 1980).

Among infections known to afflict mankind since recorded history, diarrhoeal disease caused by bacteria belonging to genus Shigella has been responsible for millions of deaths and disabilities during wars, floods, famines, and other human catastrophes (Pal, 1986). Shigellosis has a global distribution with the highest incidence in countries where hygiene and sanitation are poor (Rajasekaran et al., 1977; WHO, 1980; Pal, 1986). Man is both the reservoir and natural host of Shigella (Mossel et al., 1977; Levine, 1979). Infection is by fecal-oral route and the most common mode of spread is by person - to - person transmission (WHO, 1980). As few as 10-200 virulent organisms can produce shigellosis (Levine et al., 1973a). This represents an inoculum size 100 to 10,000 fold smaller than that believed to be required to cause illness with other known bacterial enteropathogens (Levine, 1979; WHO, 1980). As the result of the low infectious dose, bacillary dysentery is typically spread by contact transmission with infected hands (Levine, 1979).

Although reported incidence of food-borne shigellosis is very much lower than that of salmonellosis (Mossel et al., 1977), food and water-borne transmission are not uncommon (WHO,

1976; Black et al., 1978; Khan et al., 1980; Rajasekaran et al., 1977). Many outbreaks have resulted from the consumption of contaminated food, milk and water (WHO, 1976). Black et al. (1978) examined outbreaks of Shigellosis from 1961 to 1975 in the United States, and the results indicated that there were 72 Food-borne out-breaks of shigellosis during the period. Poor hygene of food handlers and inadequately chlorinated water were shown to be responsible for those outbreaks. Other studies (Raja sekaran et al., 1977; Khan et al., 1980; Pal, 1986) have shown that food- and water - borne transmission are common in developing countries owing to primitive personal hygiene and Public sanitation. According to WHO (1980), in communities with inadequate excreta disposal facilities, flies also play a significant role in transmission of shigella organism.

Most clinical and epidemiological studies (Stoll, et al., 1982; Taylor et al ., 1988; Baqui et al., 1988) indicated that Shigella species are important cause of endemic diarrhoea among children above one year old. An epidemiologic study of Sh. dysenteriae, and Sh. flexneri (Khan et al ., 1980) has found that over 60 per cent of all infections and 75 per cent of all cases belonged to the 0-9 year old age group in both Sh. dysenteriae type 1 and Sh. flexneri. The researchers suggested that the prevalence in these age group might be due to lack of attention to cleanliness, and association more closely with their peer groups and mothers.

Following ingestion of Shigella, organisms reach the intestinal lumen where they proliferate. The fundamental virulence characteristic of Shigella is its ability to invade epithelial cells and subsquently multiply (Labrec et al., 1964). After invasion of the mucosa, the proliferating Shigella elaborate an enterotoxin which is believed to have some role in initiation of diarrhoea (Keusch et al., 1972).

The predominant site of mucosal invasion and pathology includes the terminal ileum and length of the colon. Shigella multiplying in the intestinal mucosa and lamina propria stimulate a striking infiltration of polymorphonuclear leucocytes into the area of infected bowel (Levine, 1979). Death of colonic mucosal cells results in micro-ulcers through which red blood cells and plasma proteins leak into the bowel lumen. The colonic mucosa at this time is swollen, covered with mucus and marked by haemorrhages (Keusch et al., 1972).

2.3. Salmonella

The genus Salmonella is composed of motile bacteria, that conform to the definition of the family Enterobacteriaceae and the tribe Salmonellae (Edward and Ewing, 1972). Biochemical characters of the bacteria in this genus are described in detail in many text books (Edward and Ewing, 1972, Cowan and Steel, 1974; Cheesbrough, 1984). Salmonellae are grouped by their somatic (O) antigens, and serotyped on the basis of their

flagellar(H) antigens. The genus Salmonella now comprises about 2000 serotypes which can infect a wide range of warm-and cold-blooded animals (Levine,1979; WHO, 1980).

Salmonellae are among the classical enteric pathogens (Cooke, 1974; WHO, 1978). Man is the only host and reservoir of S. typhi, the agent of typhoid fever. Thus the infection is limited to man. Transmission occurs through contact with an overt case of typhoid fever or more commonly by contamination of food , water or fomites from convalescent or chronic carriers (Lubwama, 1985). Other serotypes which are associated with simple diarrhoeal disease throughout the world, such as S. typhimurium S. enteritidis, S. heideberg, S. saintpaul, S. infantis etc., are found in animals that surround man (Levine, 1979). The infected animals such as cows, chicken, turkeys, swines, pigeons, turtles are important reservoirs of infection for man (WHO,1976). According to Pether et al. (1982), the major cause of Salmonellosis includes inadequate refrigeration, preparation of food too far in advance from consumption and contaminated utensils. The source of these organisms are raw meat and poultry that enter the catering establishment. Humphery et al. (1988) studied the risk of unpasteurized milk on sale in the public, and indicated that Salmonella usually contaminate milk as the result of fecal fouling during milking. Sharp et al. (1980) reviewed a ten-year record, of milk-borne salmonellosis in Scotland and documented that at least 2,428 persons were affected by the disease as the result of consuming raw milk.

The relative frequency of salmonellosis as food-borne disease varies from country to country and depends on factors such as dietary habits, hygiene standards in food production and animal husbandry practice (WHO, 1976; WHO, 1980; Blaser, 1982). Surveillance data in USA and other industrilized countries, suggest a very high prevalence of Salmonella infection as compared to its incidence in under developed countries. In these developed countries, the surveillance data suggested that the highest incidence of salmonellosis occurs in the first year of life and more particularly in the early infancy (WHO, 1980). With regard to Salmonella infection in developing countries, due to lack of adequate surveillance, it has been difficult to evaluate the importance of Salmonellosis. Some investigators (Sanyal et al., 1977; Levine, 1979; Blaser et al., 1982) did not find Salmonellae as an important cause of infantile diarrhoea related to it's ubiquitous distribution in the tropical ecology. Others (Becker, 1960; Kagwa-Nyanzi, 1977; Lui, 1979; Al-Rajab et al., 1988) have isolated Salmonellae from infantile diarrhoeal cases and hence stress the importance of Salmonellae in infantile gastroenteritis.

Like Shigella the most important pre-requisite of virulence of Salmonella organisms is their ability to invade epithelial cells of intestinal mucosa (Levine, 1979). Strains of Salmonella typhimurium were studied, by Giannella et al. (1973) in the

ligated rabbit loop model to determine the mechanism where bacteria which invade the gastrointestinal mucosa evoke fluid secretion, and the observation suggested that mucosal invasion may be a necessary factor for intestinal fluid loss in salmonellosis. Another study by Kaura et al. (1982) indicated that both enterotoxigenicity, and invasiveness could play a vital role in the pathogensis of Salmonella diarrhoea.

2.4. Staphylococci

Staphylococci are Gram-positive cocci in pairs, tetrads or clusters. The cells show variation in size and Gram-positivity. The bacteria in this group are non-motile, non-sporing, aerobic and facultatively anaerobic, catalase positive, oxidase negative, and attack sugars by fermentation (Cowan and Steel, 1974). Staphylococci are widely distributed in the environment, in some animals, and healthy human carriers. Although the main species of medical importance is Staphylococcus aureus,other species such as Staphylococcus epidermidis, and Staphylococcus saprophyticus have also been implicated in causing disease (Breckinridges and Bergdoll, 1971; Cheesbrough, 1984).

Staph aureus produces several toxins. Ingestion of the toxins produced in foods leads to staphyloenterotoxicosis or gastroenteritis (Frazier, 1967; Mossel, 1977). The enterotoxin is so powerful in it's effect on man and causes nausea, vomiting and diarrhoea within 2-3 hours of ingestion (Girswold, 1950; Frazier, 1967; Mossel, 1977). According to Mossel (1977),

eight enterotoxins (A,B,C,C_2, D,E,F, and G) are known. They are all polypeptide chains, and show a very high degree of thermoresistance (WHO, 1976; Mossel, 1977). Although not all staphylococcal strains are capable of producing such enterotoxins, the above workers indicate that approximately 80 per cent of all strains of Staph. aureus of human origin possess the capacity of forming one or more staphyloenterotoxins.

Staphylococcal enterotoxins have been isolated from various clinical specimens (Melconian et al., 1983). They have been isolated from lesions of hospital patients, healthy carriers, food stuffs, and milk (Wieneke, 1974; Reali, 1982; Sharp, 1989). Staphylococcal food-borne gastroenteritis has been documented in many parts of the world. Earlier studies in the United States (Feig, 1950) showed that 80 per cent of all reported bacterial outbreaks of gastroenteritis belonged to staphylococcal enterotoxin. Holmberg and Blake (1984) reviewed all outbreaks in the United States for the period 1977 through 1981, and the work indicated that there were 131 staphylococcal food-borne outbreaks which affected 7,126 persons. These investigators and others (Girswold, 1950; Mando-Khot et al., 1983) have elucidated that improper storage, contaminated equipment, poor hygiene of food-handlers, contributed a major part to the outbreaks. Steenberg et al. (1983) examined children's dishes and drinking water, in a rural area of Kenya and found that 12 per cent of the dishes

harboured Staph aureus at levels above the threshol of acceptability.

According to Meyer (1953) the mere finding of an enterotoxin producing strains of staphylococci is not sufficient to cause the food poisoning. The viable count of staphylococci in the examined food is essential. Appreciable levels of enterotoxins are produced only after a considerable growth of the Staphylococcus.. Many investigators (Frazier, 1967; WHO, 1976; Mandakhot et al.,1982) indicated that a population of 10^5 to 10^9 per milliliter or gram of food must be attained to produce appreciable quantities o[illegible]rotoxin.

MATERIALS AND METHODS

1. Subjects

A total of 244 babies who were brought to five clinics and two hospitals in Addis Ababa, were included in the study from January 1989 to November 1989. The subjects had varying complaints such as fever, cough, and diarrhoea, while some were brought for clinical check-up and routine immunization. The criteria for selecting the babies for the study were:

1. Bottle-feeding babies,
2. Possession of the bottles by the mothers at the time of the hospital or clinic visit,
3. The presence of adequate volume of feeds in the feeding bottles,
4. The willingness of the nursing mother to respond to the questionnaire and give samples needed for the study.

The mothers were interviewed in the waiting rooms of the hospitals/clinics and questionnaires were filled by the author. The questionnaire used in the study is seen in the Appendix section.

2. Collection and Processing of Specimens

2.1 Bottle - Contents

About 10 ml of the bottle-contents were carefully transferred into sterile screw capped containers. The samples were then transported to the laboratory within 1-2 hours time.

The average time between collection of the bottle-contents and processing was 2 hours. The samples were kept in a refrigerator at $4^{o}C$ until processing. The refrigerated samples were brought to room temperature before inoculation into differnt media. The bottle - contents were serially diluted up to 10^{-5}, for aerobic plate count using normal saline solution (Banwart, 1983). Then a volume of 1ml of each serial dilution was pipetted into sterile petridishes, and tryptone glucose extract agar prepared from Difco ingredients (ISOM, 1976) was poured on to the culture plates. Then the content was mixed by rotating the petridishes gently. The agar was allowed to solidify at room temperatures, and incubated aerobically at $37^{o}C$ for 48-72 hours.

For direct culture procedure, MacConkey, Salmonella - Shigella, Xylose lysine deoxycholate (XLD), Mannitol salt, and sheep blood agars prepared from Difco powders were inoculated with the bottle - contents using sterile pasteur pipettes. This was for isolation of enteric pathogens. The culture plates were then incubated for 24-48 hours at $37^{o}C$. Furthermore, 2.5 ml of the samples were removed with sterile pipettes from the original samples and passed into lactose broth as suggested by ISOM (1976) for pre-enrihment of Salmonella species in heat treated food materials. Another 2.5 ml of the samples were also tranferred into Tryptone - Soya Yeast broth (TSY) for enrichment of other pathogens. After an over night incubation, 2.5 ml. of the pre-enrichment culture was transferred into tetrathionate enrichment

broth as recommended by previous workers (ISOM, 1976; Mossel, 1977). This was for the recovery of Salmonella in heat treated foods. The broth cultures were incubated at 37°C for 48-72 hours, after which inocula were taken with pasteur pipettes and ioculated into the primary isolation media indicated above in the direct culture procedure.

2.2 Teats

In addition to sampling bottle-contents, teats of each feeding-bottle were sampled by rubbing the external surface of the teats with wet cotton-tipped swabs. The teat swabs were then dipped into nutrient broth. These were carried to the laboratory along with other specimens and kept at 4°C in the refrigerator until processing. The culture media and the bacteriological techniques employed for processing the teat-swabs were similar to that described for the bottle-contents, except that the teat-swabs after direct inoculation of the primary isolation media, were left dipped in the original nutrient broth and incubated for an over night at 37°C. About 2ml volume of the over night broth culture were pipetted into tetrathionate, and TSY broth enrichments. The enrichment cultures were treated as described above for the bottle contents. After 48-72 hours of incubation the enrichment cultures were sub-cultured into MaCconkey, Salmonella-Shigella, Mannitol salt, and Blood agars.

2.3 Fecal Specimens

Fecal samples were collected from each bottle-fed baby selected for the study. This was done by inserting sterile cotton-swabs into the rectum of each baby. The swabs were then immersed into Carry-Blaire transport media (Difco). These specimens were transported to the laboratory within 2 hours time. The primary isolation media used for culturing the fecal samples were similar to those described above. After inoculation of the primary culture media, the rectal-swabs were immersed in Kauffmann enrichment broth and incubated for 24 hours at 37 C as described by Edward and Ewing (1972). Then, sub - culture was made from the Kauffmann broth into Salmonella-Shigella and MacConkey agars. These were incubated for another 24 hours, and examined to do necessary bacterial identification of pathogens.

3. Total Colony Counting and Identification of Bacteria

Colonies on tryptone glucose extract agar plates were counted with the aid of colony counter equipped with a guide plate ruled in square centimeters. Plates with 30-300 colonies were selected and counted, and then the count per ml for each dilution was multiplied by the dilution used. Colonies on MacConkey and Salmonella - Shigella agars were differentiated morphologically and on the basis of fermentation reaction as lactose fermenting or non-lactose fermenting colonies. Bacterial

growth on Blood agar was observed and differentiated as haemolytic or non-haemolytic colonies. Growth on mannitol salt agar was identified as mannitol fermenting or non-mannitol fermenting colonies.

3.1. Biochemical and Serological Identification of Salmonella and Shigella

A single non-lactose fermenting colony was picked from the MacConkey agar/Salmonella - Shigella agar and inoculated with the use of sterile straight wire loop into a 3 ml nutrient broth. This was incubated at 37 °C for 2 to 4 hours until growth was ascertained by turbidity. The broth was then used to inoculate the standard set of bio-chemical testing media. The biochemical testing media included: Kligler iron agar , lysine iron agar, motility medium, Simmon's citrate, glucose peptone water, urea agar slant, and other media described by Edward and Ewing (1972). All the biochemical tubes under test were examined after incubation at 37 °C for 24 hours. Those bacteria which fully conform to the biochemical characteristics of Salmonella, and Shigella were further confirmed by serological tests with the respective antisera obtained from Difco laboratories.

3.2 Identification of Enteropathogenic Escherichia Coli

Three to four lactose fermenting *E. coli* like colonies were picked from MacConkey agar, and each colony was inoculated into a 3 ml nutrient broth with the use of a sterile straight wire loop. This was incubated at 37 °C for 2-4 hours and growth was acertained by turbidity of the culture media. Then the broth culture was used to inoculate the standard biochemical testing media described in Section 3.1 above. Organisms that conformed with the biochemical characteristics of *E. coli* were seeded on blood agar for serological testing. Colonies from blood agar were tested by slide agglutination technique using polyvalent *E. coli* antisera. Those giving positive results with the polyvalent antisera were further tested with the monovalent *E.coli* antisera. The antisera were obtained from Difco laboratories.

3.3. Identification of Other Bacteria

Coliform organisms, and other members of *Enterobacteriaceae*, were identified by the biochemical tests to genus or species level. Gram positive bacteria such as *Staphylococcus* and *Bacillus* species were identified with combination of Gram's strain, catalase and coagulase tests, and whenever necessary by using appropriate biochemical tests described by Cowan and Steel (1974).

4. Drug Susceptibility Test

Antimicrobial susceptibility test was performed for enteropathogenic bacteria isolated from the various specimens. Disc diffusion technique previously described by Bauer et al.(1966) were employed. Antimicrobial discs produced by BioMerieux were used in the antimicrobial susceptibility test. These were ampicillin (10 mcg*), cephalothin (30 mcg), kanamycin (30 mcg), gentamicin (10 mcg), streptomycin (10 mcg), polymixin B(300 mcg) trimethoprim - sulfamethoxazole (23+1.25 mcg), chloramphenicole (30 mcg), vancomycin (30 mcg) erythromycin (15 mcg), clindamycin (2 mcg), penicillin (10 unit), sulfadiazine (250 mcg), and methicillin (5 mcg). The test organisms were grown in tryptone soya yeast broth, and Muller-Hinton agar plates were swabbed with the bacterial cultures from the tryptone soya yeast broth. Then the Muller-Hinton agar was allowed to dry at room temperature for 15-20 minutes, and a set of discs (11 discs for testing Staph aureus, and Shigella isolates, and 10 for testing EPEC was placed on the culture plates with the aid of an automatic dispenser (BBL). The culture plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured in millimeter using a graduated caliper. The size of inhibition zones were interpreted as sensitive, intermediate, and resistant. The intermediate strains were included with the sensitive strains for convenience since they were relatively few. Standard reference strains of Escherichia coli (ATCC** 25922), and Staphylococcus aureus (ATCC 25923) were used for quality control of the test.

5. Detection of Beta-lactamase in Staphylococcus aureus:

All penicillin resistant Staph. aureus strains were tested for Beta-lactamase production. Cefinase strips (BBL) were used for detection of Beta-lactamase producing Staph aureus. The strip was placed on a microscope slide and moistend with a drop of distilled sterile water. Then, a loopful of Staph. aureus colonies from blood agar, was smeard on to the disc surface. Positive reaction was acertained by development of red colour on the area where the culture was applied, in about 5 - 60 minutes time, while negative for Beta - lactamase production was confirmed by retaining the yellow color of the cefinase strip.

N.B * mcg = microgram
** ATCC = American type culture collection

RESULTS

1. Background Information from Questionnaire

A total of 249 mothers were interviewed in an eleven months (January - November 1989) study period. Of these, 244 (97.9%) responded to questions posed in the questionnaire and provided samples of bottle - contents for bacteriological analysis. In addition, stool samples were collected from the infants for necessary bacteriological analysis.

Table 1 shows the educational status of the mothers. It can be seen from the Table that 233 (95.5%) mothers had at least a primary school education, and only 11(4.5%) were illiterate. Table 2 shows the occupational status of the mothers. Only 68(27.9%) were government employees, while 158 (64.7%) were housewives. Out of 244 mothers, 242(99.1%) indicated that they get water from piped sources (data not shown in any of the Tables). However, most of these mothers explained that they collect water from out-door pipes either neighbours' or municipal posts at some distance from their houses.

Table 3 shows the types of food in bottles usually given to the babies. Of the total mothers, 151 (61.9%) feed their babies with cow's milk, and the remaining 93 (38.1%) feed with different food types listed in the Table. All the mothers indicated

Table 1 Educational Background of Nursing Mothers

Level of Education	number of mothers	%
Illiterate	11	4.5
Primary School (grades 1-6)	118	48.4
Secondary School (grades 7-11)	36	14.8
12 th Grade and above	79	32.4
Total	244	100

Table 2. Occupational Status of Mothers

Occupation	Number	%
Government employee	68	27.9
Private employee	18	7.4
House - wife	158	64.7
Total	244	100

Table 3 Type of Bottle - contents Analysed

Bottle - content	Number	%
Cow's milk**	151	61.9
Cereal blend	17	6.9
Commercial milk powder*	33	13.5
Mixture of cereals and cow's milk	28	11.5
Others (tea, water, etc)	15	6.1
Total	244	100

* = Dehydrated canned milk powder

** = Fresh or pasteurized milk bought from local market

that they prepare infant foods by boiling and the length of time that infant foods are usually keept after preparation is shown in Table 4. A total of 207(84.8%) of the mothers keep feeds for longer than half a day (> 6 hours).

Table 5 shows the method of handling care of the feeding bottles and teats. As it can be seen from the Table, 191(78.3%) mothers boil, 35 (14.3%) soak in hot water and 18(7.3%) wash the feeding bottles and teats with water and soap. The frequency of washing bottles and teats is presented in Table 6. Out of 244 mothers, 179(73.3%) wash the feeding utensils once or twice within 24 hours. There is no significant association between the methods of handling care of bottles and the educational level of mothers (X^2 test, $P > 0.05$).

The age group of the bottle- fed babies in the study is shown in Figure 1. The babies age ranged from less than 4 months to greater than 24 months. Out of the 244 babies, 62(25%) had diarrhoeal problems, and were diagnosed by physicians to have acute gastroenteritis. The remaining 182(74.6) included babies with different other complaints, and those attending the hospitals/clinics for routine immunization or check-up. Of the babies with gastroenteritis 22(35.5%) were in the age group between 5 and 9 months, and 50(27.5%) babies without gastroenteritis were in the same age group as above.

Table 4 Educational Status of Mothers Versus Duration of Feeds Kept from Preparation Until Consumption

Length of time (hours)	Illiterate		Primary School		Secondary School		12th and above		Total	
	No.	%	No.	%	No.	%	No	%	No.	%
Less than 1	0	0	4	3.4	1	2.8	3	3.8	8	3.2
1 – 3	0	0	6	5.1	2	5.6	8	10.8	16	6.5
4 – 6	2	18.2	6	5.1	3	8.3	2	2.5	13	5.3
> 6	9	81.8	102	86.4	30	83.3	66	83.5	207	84.8
Total	11	100.0	118	100.0	36	100.0	79	100.0	244	100.0

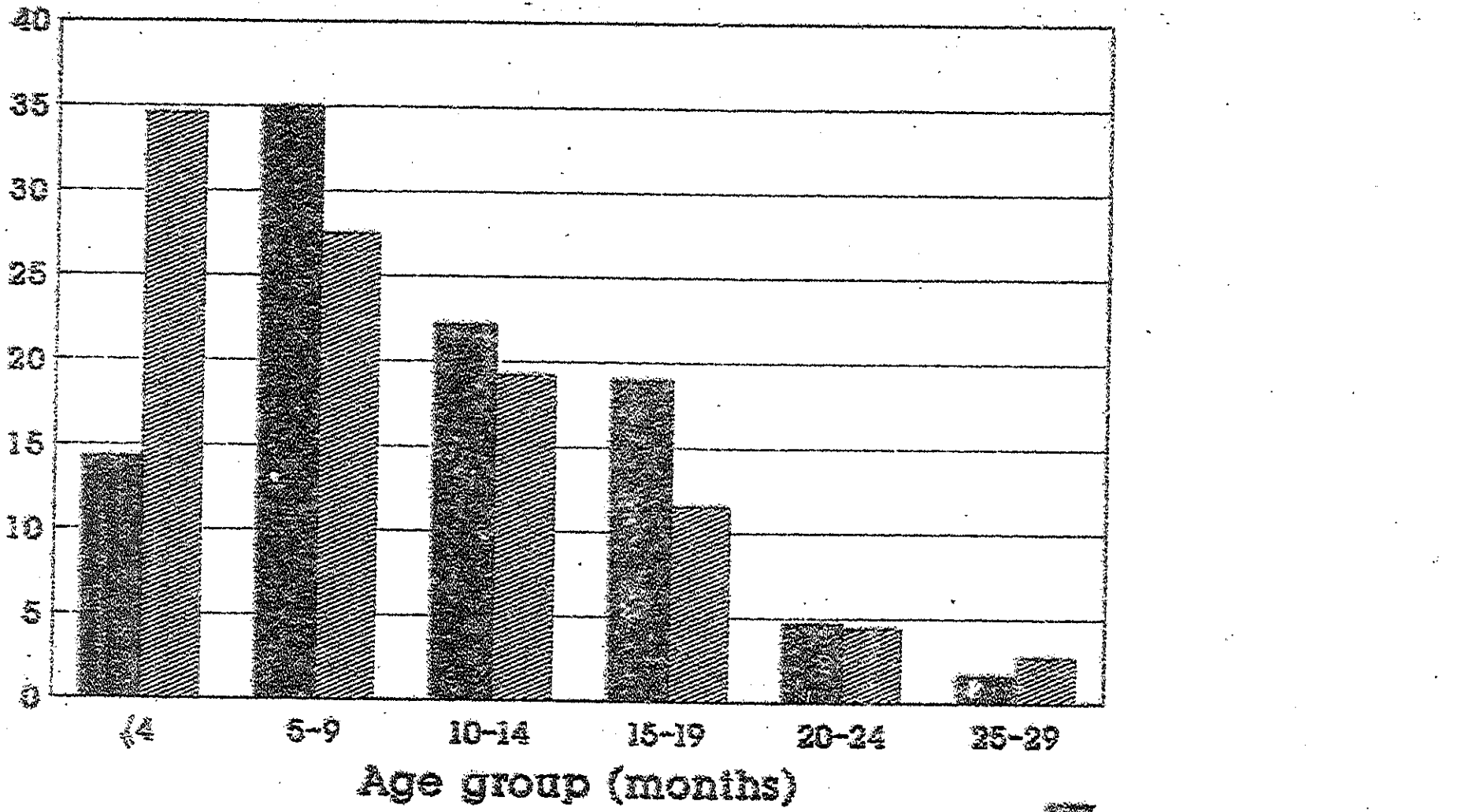

Fig.1 Age - group of bottle - fed babies in the study

* Percentage is out of 62 babies with gastroenteritis, and 182 non-gastroenteritis cases

2. Bacteriological Analysis of Samples

A total of 549 samples, which included 244 bottle - contents, 244 rectal - swabs, and 61 teat - swabs were collected and cultured for bacteriological analysis. Table 7 shows the various bacterial species isolated from different bottle - contents. As it can be seen from the Table, 270 bacterial strains were isolated from a total of 244 samples of bottle - contents. The highest bacterial yields were obtained from cereal samples. A total of 26 bacterial strains were recovered from 17 different samples of cereal blends (152.9% yield). Cow's milk samples yielded 165 different bacterial strains. Some samples of the bottle-contents yielded more than one bacterial species. Of the 270 bacterial isolates, 63 (23.3%) were *E. coli*; and of these *E. coli*, 9(14.2%) were the classically recognized serotypes of enteropathogenic *E. coli* (EPEC). Five of the EPEC were detected from cow's milk samples, and the remaining 4 were isolated from samples of commercial powder milk. The serotypes of EPEC isolates included O127:K63(B 8), O126:K 71 (B 16) O119:K 69 (B14), O28:K 73 (B 18), and O55:K 59 (B5). Some of these serotypes were also isolated from rectal - swab cultures. Serotypes, O114:K 90 (B-), and O55:K 59 (B 5) were detected only from bottle - contents (Table 8).

Table 7. Bacterial Isolates from Different Samples of Bottle - Contents.

Bacterial isolate	Type of bottle - contents					
	Cow's milk (151)		Comm* milk (33)		Cereal (17)	
	No	%	No	%	No	%
Enteropathogenic E. coli	5	3.0	4	11.4	0	0
E. coli type I	30	18.8	4	11.4	5	19.2
Other E. coli***	5	3.0	0	0	1	3.8
Shigella flexneri	1	0.01	0	0	0	0
Staphylococcus aureus	5	3.0	1	2.8	1	3.8
Bacillus spp.	6	3.6	0	0	0	0
Enterobacter spp.	29	17.6	12	34.2	11	42.3
Citrobacter spp.	25	15.5	1	2.8	2	7.6
Klebsiella spp.	35	21.1	6	17.1	1	3.8
Proteus spp.	2	1.2	0	0	0	0
Acinetobacter spp.	12	7.2	3	8.5	1	3.8
Pseudomonas spp.	2	1.2	2	5.7	0	0
Streptococcus spp.	4	2.4	1	2.8	2	7.6
Other Organisms (Yeasts, unidentified spp)	4	2.4	1	2.8	2	7.6
Total	165	100	35	100	26	100

* Commercial powder milk
*** Biochemically conform to E. coli characteristic but do not aggultinate EPEC antisera

Table 7 (Continued)

Bacterial isolate	Type of Bottle-content			
	Cow's milk + cereal (28)		Others** (15)	
	No	%	No	%
Enteropathogenic E. coli	0	0	0	0
E. coli type I	5	17.8	1	6.3
Other E. coli**	3	10.7	0	0
Shigella flexneri	0	0	0	0
Staphylococcus aureus	1	3.5	1	6.3
Bacillus spp.	0	0	0	0
Enterobacter spp.	7	25	7	43.8
Citrobacter spp.	3	10.7	0	0
Klebsiella spp.	6	21.4	2	12.5
Proteus spp.	0	0	1	6.3
Acinetobacter spp.	1	3.5	1	6.3
Pseudomonas spp.	0	0	0	0
Streptococcus spp.	2	7.1	0	0
Other Organisms (yeasts, unidentified spp. etc)	0	0	3	18.8
Total	28	100	16	100

** = tea, water, oral rehydration solution etc.

Table 7 (Continued)

Bacterial isolate	Total	% of total
Enteropathogenic E. coli	9	3.3
E. coli type I	45	16.7
Others E. coli	9	3.3
Shigella flexneri	1	<0.01
Staphylococcus aureus	9	3.3
Bacillus spp.	6	2.2
Enterobacter spp.	66	24.4
Citrobacter spp.	31	11.5
Klebsiella spp.	50	18.5
Proteus spp.	3	1.1
Acinetobacter spp.	18	6.6
Pseudomonas spp.	4	1.4
Streptococcus spp.	9	3.3
Other organisms (yeasts, unidentified species)	10	3.7
Total	270	100

Table 8. Serotypes of Enteropathogenic E. coli isolated from Different Specimens

Serotypes	Isolates from Rectal - Swabs number	%	Isolates from Bottle - contents number	%	Total No.	%
0127:K 63 (B 8)	3	13.0	1	11.1	4	12.1
0126:K 71 (B 16)	3	13.0	1	11.1	4	12.1
0125:K 70 (B 15)	2	8.7	1	11.1	3	9
0124:K 72 (B 17)	1	4.3	0	0	1	3
0128:K 67 (B 12)	3	13.0	0	0	3	9
0111:K 58 (B 4)	3	13.0	0	0	3	9
044 :K 74	2	8.7	0	0	2	6
026:K 60(B 6)	1	4.3	0	0	1	3
0119:K 69(B 14)	1	4.3	1	11.1	2	6
028:K 73 (B 18)	2	8.7	2	22.2	4	12.1
078:K 80 (B)	1	4.3	0	0	1	3
018:K 77 (B 21)	1	4.3	1	11.1	2	6
0114:K 90 (B)	0	0	1	11.1	1	3
055:K 59(B 5)	0	0	1	11.1	1	3
Total	23	100	9	100	32	100

Shigella was isolated from only one sample. Staphylococcus aureus constituted 9(3.3 %) of the total bacterial isolates. Of the 9 Staph aureus isolates, 5(55.5%) were detected from cow's milk and the remaining 4(44.4%) were from other bottle - contents. No Salmonella or Vibrio species were detected in any of the bottle - content samples. The remaining group of bacterial isolates were predominantly the coliform group which included, Enterobacter spp., 66 (24.4 %), Klebsiella spp. 50(18.5%), and Citrobacter spp. 31(11.5%). Other bacterial species such as Pseudomonas spp.,Proteus spp.,or Streptococcus spp. were only isolated infrequently. Bacillus cereus was isolated from only one sample of bottle - content (Table 7).

Table 9 presents data on bacterial isolates during different seasons of the year. As it can be seen from this Table, the isolation rate of the different bacterial strains appeared to be higher in the rainy season than in the dry season. Of the samples collected during the rainy season (June-October), 6(4.7%) yielded EPEC. The corresponding yield of EPEC from samples collected during the dry season (November-April) was only 3 (2.6%). Staph aureus was isolated from 7 (5.4%) of the total samples collected during the rainy seasons, and 2 (1.7%) of the dry season. Similarly, Klebsiella spp. and Enterobacter spp. were isolated in higher rate during the rainy season than the dry season.

Table 9. Seasonal Isolates from Feeding Bottle - contents

Bacterial isolate	Dry season (November - April) N* = 115		Rainy season (June - October) N = 129	
	No.	%	No.	%
Staph. aureus	2	1.7	7	5.4
Shigella spp.	0	0	1	0.01
Enterobacter spp.	11	9.5	55	42.6
Enteropathogenic E. coli	3	2.6	6	4.7
E. coli type I	15	13.0	29	22.5
Citrobacter spp.	17	14.8	14	10.9
Klebsiella spp.	15	13.0	35	27.1
Others **	45	39.1	15	11.6
Total	108	100	162	100

* Number of sample analysed

** Others = Non coliform group of bacteria listed in Table 7

The over all rate of isolation of bacteria in the rainy season was 125 % (162/129), and 93.9 % (108/115) in the dry season (Table 9). The association between isolation frequency of different bacteria and seasons in which the samples were collected appears to be statistically significant (χ^2 test, $P < 0.01$)

Figure 2 shows total bacterial count per ml of samples. Of the total samples of bottle contents, 144(59%) harboured more than 10^6 colonies of bacteria per ml of sample. Cereal samples or mixed preparation of cereals and cow's milk appeared to be grossly contaminated. Bacterial counts greater than 10^6 colonies per ml of sample were obtained from 11(64.7%),and 18(64.3%) of cereal, and mixture of cereal and cow's milk samples respectively. Similarly, 88(58.3%) of cow's milk and 16(48.5%) of commercial milk powder samples harboured counts greater than 10^6 colonies of bacteria per ml of sample (Table 10). The association between the type of bottle - contents and bacterial count per ml of smaples is not statistically significant (X^2 test, $P > 0.05$).

Total bacterial count versus method of handling care of feeding bottles is shown in Table 11.

Table 10. Bacterial Count per ml of Bottle - contents

Bacterial count/ml	Type of bottle - contents									
	Cow's milk		Comm. powder milk		Cereal		Cereal + cow's milk		Others*	
	No	%	No	%	No	%	No	%	No	%
$< 10^4$	11	7.3	2	6.1	0	0	0	0	2	13.3
$2\times10^4 - 10^5$	25	16.6	5	15.2	2	11.8	2	7.1	1	6.7
$2\times10^5 - 10^6$	27	17.9	10	30.3	4	23.5	8	28.6	6	40.0
$> 10^6$	88	58.3	16	48.5	11	64.7	18	64.3	6	40.0
Total	151	100	33	100	17	100	28	100	15	100

* other = tea, water, etc

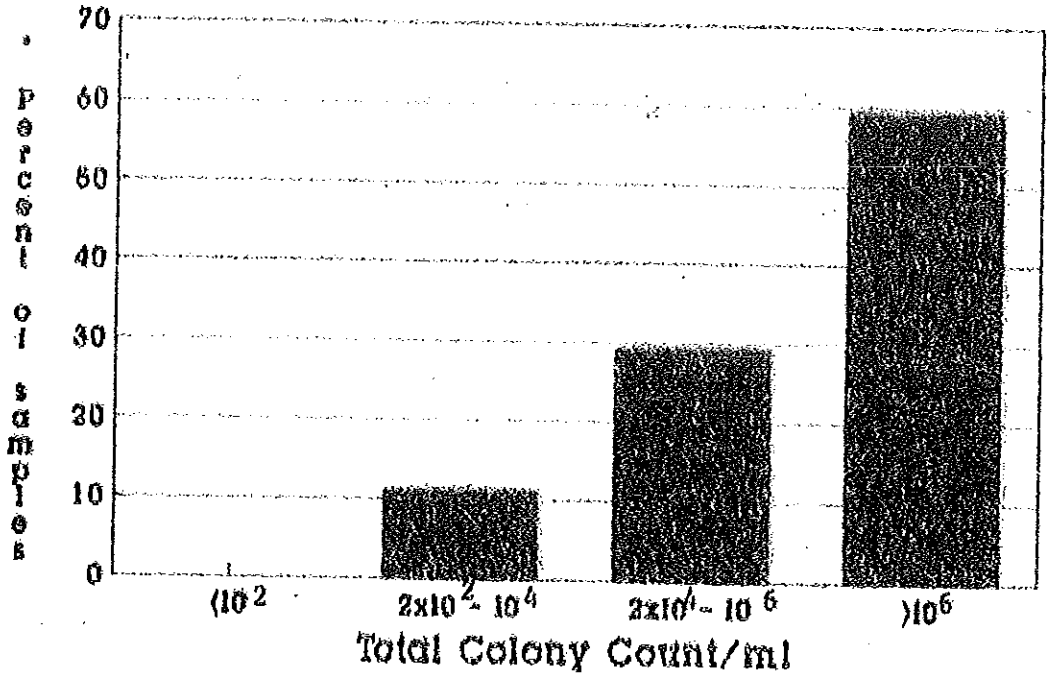

Fig. Percent of samples of bottle-contents versus bacterial count.

* Percent is out of 244 bottle - content samples.

Table 11 Bacterial Count Versus Method of Handling Care of Bottles

Range of Bacterial count/ml.	Method of handling care ***					
	Boiling		Soaking *		Washing **	
	No.	%	No.	%	No.	%
$10^2 - 10^4$	39	20.4	8	22.8	1	5.9
$2x10^4 - 10^6$	38	19.8	13	37.1	9	50.0
$>1x10^6$	114	59.7	14	40.0	8	44.0
Total	191	100	35	100	18	100

* = Soaking in hot water
** = Washing with unboiled water and soap
*** = Information obtained by interviewing mothers; and duration of treatment, temperature, etc are not known.

The count of most samples taken from bottles, 114(59.7%) boiled, 14(40.0%) soaked in hot water, and 8(44.0%) washed in unboiled water and soap, contained counts more than 10^6 bacterial colonies per ml of samples. There is no significant association between the bacterial load in the bottle-contents and the various method of handling care of feeding bottles, used in the studied population (X^2 test, $P > 0.05$). Figure 3 shows bacterial counts per ml of samples in different seasons. It appears that the bacterial load per ml of samples in the rainy months of the year are slightly higher than the load in the dry months. For instance all the counts recorded from samples collected in the months of January and February were 10^6 colonies per ml. or below, whereas in the months of June and July 14(12.0%), and 25(21.4%) of all samples analysed respectively, had bacterial counts greater than 10^6 colonies per ml. of samples.

Bacterial isolates from teat-swabs are listed in Table 12. As in the case of bottle - contents, the most frequent isolates from teat - swabs included coliforms in the following order, Enterobacter, Citrobacter, klebsiella. Figure 4 compares bacterial isolates detected from bottle-contents and samples of teat-swabs.

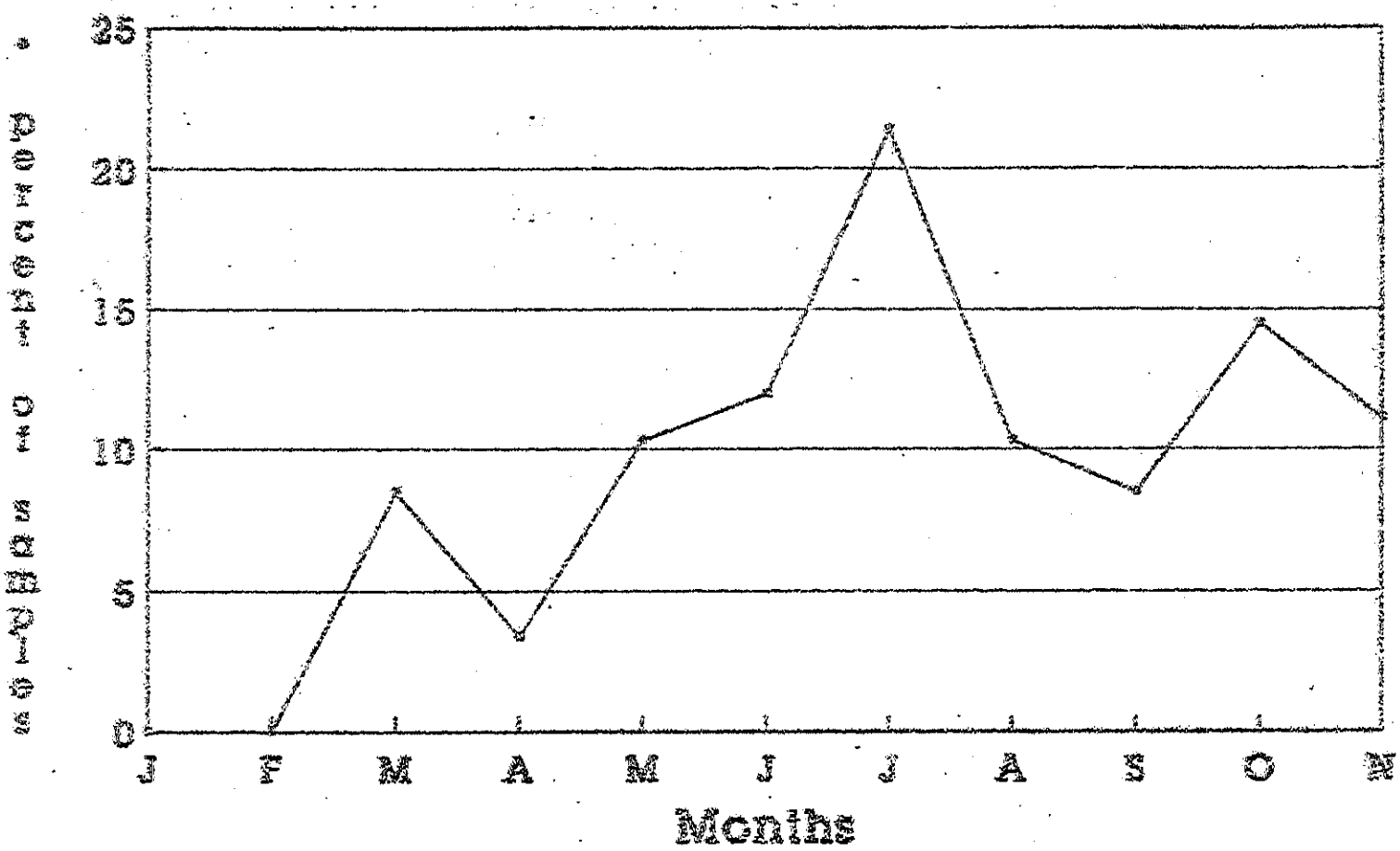

Fig.3 Per cent of samples of bottle-contents having bacterial counts more than 10^5 in each month (Jan.89-Nov.89)

* - Percentage is out of total samples collected each month.

Table 12. Bacterial Isolates from Teat - Swab Samples

Bacterial isolates	Number	%
Enteropathogenic E.coli	1	1.2
E. coli type I	7	8.4
Other E. coli *	5	6.0
Enterobacter spp.	20	24.0
Klebsiella spp.	10	12.0
Citrobacter spp.	11	13.2
Shigella dysenteriae	2	2.4
Staphylococcus aureus	10	12.0
Bacillus spp.	1	1.2
Acinetobacter spp.	10	12.0
Streptococcus spp.	1	1.2
Psendomonas spp.	2	2.4
Others **	3	3.6
Total	83	100

* = Biochemically E. coli but negative with antisera for EPEC

** = Yeasts, and unidentified bacterial species.

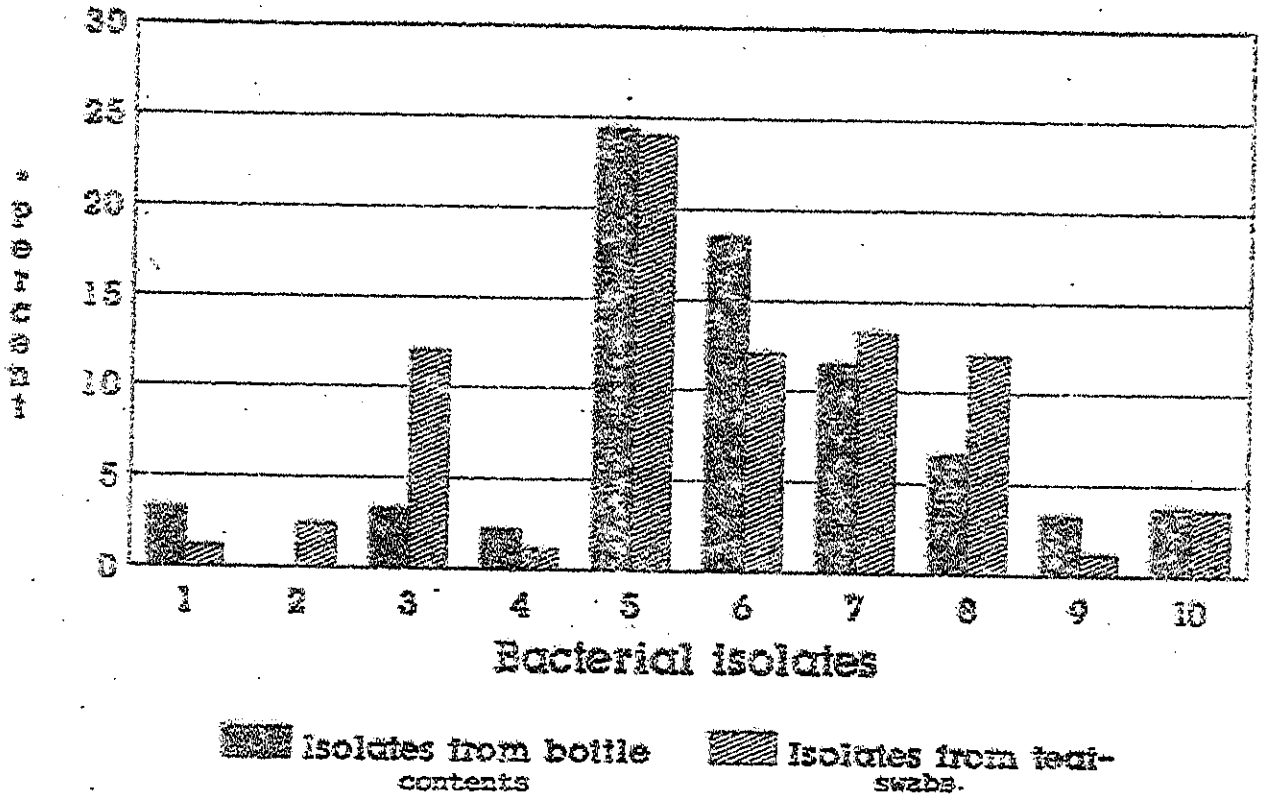

Fig. 4 Bacterial isolates from bottle contents and teat-swab

1. EPEC 2. Shigella spp., 3. S aureus 4. Bacillus spp., 5. Enterobacter spp., 6. Klebsiella spp., 7. Citrobacter spp., 8. Acinetobacter spp., 9. Streptococcus spp., 10. Others (Pseudomonas, Proteus, etc)

* - Percentage is out of 270 total isolates for bottle-contents, and out of 83 for teat-swabs.

Shigella was detected from 2(3.2%) teat-swabs, and EPEC from only 1(1.6%) teat samples.

Table 13 presents enteric pathogens isolated from 28(11.4%) of 244 rectal-swab cultures. Out of 62 rectal-swabs collected from babies with gastroenteritis, 10(16.4%) yielded enteric pathogen. Only in one case were two enteropathogens isolated from a rectal swab-culture. Of the rectal-swabs from babies without gastrointestinal complaints, 18 (9.9%) yielded enteric pathogens listed in Table 13. The isolation rate of enteric pathogens from gastroenteritis cases is not significantly greater than the rate of isolation of these pathogens from non-gastroenteritis stool samples (Z - test, P>0.05). EPEC was the predominant isolate from both group of babies (Table 13). It was isolated from 9(14.5%) of the 62 rectal swabs collected from babies with gastroenteritis, and 14(7.7%) of the 182 rectal-swabs from babies without gastrointestinal illness. The isolation rate of EPEC from the former group is significnatly higher than the rate from the latter group (Z-test, P<0.05). The frequent EPEC serotypes included O128:K 67 (B12), O127:K 63(B 8), O126:K 71(B 16), O111:K 58(B 4), and O44:K 74. Most of the serotypes from rectal-swabs of gastroenteritis cases were identical to those serotypes detected from non-gastroenteritis samples (Table 8). However, certain serotypes: O125:K 70(B 15), 124:K 72 (B 17), O119:K 69(B 14), O26:K 60(B 6), and O28: K 73(B 18), were isolated only from the rectal-swabs of non-gastroenteritis babies.

Table 13. Enteric Pathogens from the Stool of the Bottle-fed Babies

Isolates	Gastroenteritis cases (Samples = 62)		Non-gastroenteritis cases (Samples = 182)	
	number	%	number	%
EPEC	9	14.5	14	7.7
Sh. boydii	1	1.6	1	0.01
Sh. flexneri	1	1.6	0	0
S. aureus	0	0	3	2.2
Total	11	17.7	18	9.9

In only three instances were EPEC isolated from stool samples of the babies and at the same time from their feeding bottles; and the serotypes were identical in only one case. This particular serotype was isolated from a baby with gastrointestinal complaints. Shigella was detected only from 3 (1.2 %) of the 244 rectal-swab cultures. Of these Shigella isolates 2 were from babies with intestinal illness, and the remaining 1 was from a baby without gastroenteritis. Staphylococcus aureus was isolated from 3(1.2%) of the 244 stool samples, and all of these isolates were detected from non-gastroenteritis babies. No Salmonella, or Vibrio species were detected in any of the rectal-swabs.

Antimicrobials and abbrevations used in this paper for the antimicrobial drugs are shown in Table 14. The antimicrobial susceptibility test result of EPEC strains is presented in Table 15. As can be seen fro the Table, 24(92.3%) of the EPEC isolates in this study, were sensitive to kanamycin. Similarly, all isolates of EPEC tested were sensitive to gentamicin. Other anti-bacterial agents to which 15(57.6%), 18(69.2%) 22(84.6%), and 20(76.9%) of EPEC strains respectively sensitive were carbenicillin, trimethoprim-sulfamethoxazole, Polymixin B, and chloramphenicole. Of the EPEC isolates 15(57.7%), 14(53.8%), 11(42.3%), and 9(34.6%), were respectively resistant to tetracycline, ampicillin, streptomycin and carbenicillin (Table 15).

Table 14. Abbrevations of Antibacterial Agents Used in This Paper

Abbrevation	Full name
CF	Cephalothin
Te	Tetracycline
Am	Ampicillin
CB	Carbenicillin
K	Kanamycin
Gm	Gentamicin
PB	Polymxin B
S	Streptomycin
SxT	Trimethoprim-sulfa methoxazole
C	Chloramphenicol
Cc	Clindamycin
P	Penicillin
Va	Vancomycin
E	Erythromycin
Dp	Methicillin
G	Sulfadiazine

Table 15. Drug Susceptibility of Enteropathogenic E. coli

(Number of isolates tested = 26)

Antibacterial Agents	Sensitivity		Resistance	
	number	%	number	%
Cephalothin	17	65.4	9	34.6
Tetracycline	11	42.3	15	57.7
Ampicillin	12	46.2	14	53.8
Carbenicillin	15	57.6	11	42.3
Kanamycin	24	92.3	2	7.7
Genatamicin	26	100	0	0
Streptomycin	15	57.6	11	42.3
Polymxin B	22	84.6	5	19.2
Trimethoprim - sulfamethoxzole	18	69.2	8	30.8
Chloramphenicol	20	76.9	6	23.1

Drug susceptibility test results of Shigella isolate are shown in Table 16. Of the antibacterial drugs, kanamycin, gentamicin, trimethoprim - sulfamethoxazole, chloramphenicol, and polymixin B were effective against all the Shigella isolates. Of the 6 Shigella strains tested, 5 (83.3%) were resistant to ampicillin, and carbenicillin. Similarly, 4(66.7%) 3(50%), and 3(60% of Shigella tested) were resistant to cephalothin, tetracycline, and sulfadiazine respectively (Table 16). Drug susceptibility test result of Staph. aureus isolates is shown in Table 17. As it can be seen from the Table, 18(94.7%), 16(84.2%), 14(73.7%), and 14(73.7%) of Staph. aureus isolates were sensitive respectively to gentamicin, kanamycin, clindamycin, and cephalothin. Other antibacterial agents which were effective against 12(63.2%) or more of Staph. aureus isolates were erythromycin, chloramphenicol, and streptomycin. A total of 17(89.5%) of Staph. aureus isolates were resistnat to penicillin. Similarly, 14(73.7%) of Staph. aureus strains were resistant to tetracycline. All penicillin resistant strains were found to be B-lactamase producers.

Table 18 presents data on single and multiple resistance of the bacterial isolates from various samples. All of the 19 Staph. aureus isolates and 22(84.6%) of EPEC were resistant to one or more antibacterial drugs.

Table 16. Drug Susceptibility of *Shigella* spp. isolated from Various Specimens (number of isolates = 6)

Antibacterial Agents	Sensitivity		Resistance	
	number	%	number	%
Cephalothin	2	33.3	4	66.7
Tetracycline	3	50.0	3	50.0
Ampicillin	1	16.7	5	83.3
Carbenicillin	1	16.7	5	83.3
Gentamicin	6	100	0	0
Kanamycin	6	100	0	0
Streptomycin	5	83.3	1	16.7
Polymxin B	6	100	0	0
Trimethoprim - sulfamethoxazole	6	100	0	0
Chloramphenicol	6	100	0	0
Sulfadiazine*	2	40	3	60

* - only 5 *Shigella* strains were tested with sulfadiazine

Table 17. Drug Susceptibility of *Staphylococcus aureus* Isolated from Various Specimens (number tested = 19)

Antibactrial Agent	Sensitivity		Resistance	
	number	%	number	%
Clindamycin	14	73.7	5	26.3
Penicillin	2	10.5	17	89.5
Cephalothin	14	73.7	5	26.3
Tetracycline	5	26.3	14	73.7
Kanamycin	16	84.2	3	15.8
Gentamicin	18	94.7	1	5.3
Streptomycin	13	68.4	6	31.6
Chloramphenicol	12	63.2	7	36.8
Vancomycin	11	57.9	8	42.1
Erythromycin	12	63.2	7	36.8
Methicillin	9	47.4	10	52.6

Like wise, 15(78.9%), 17(65.3%), and 5(83.3%) of Staph. aureus, EPEC and Shigella spp. were respectively resistant to three or more antibacterial agents. Out of 51 bacterial isolates tested, 8(15.7%) were resistant to three antibacterial drugs. Similarly, 9(17.6%), 8(15.7%), 3(5.8%), and 2(3.9%) of the bacterial isolates were resistant to four, six, seven, and eight antibacterial agents respectively. Only one (1.9%) ofStaph. aureus isolate was resistant to nine of the 11 antibacterial drugs.

The antibiograms of resistance of EPEC are shown in Table 9. The pattern of resistance of EPEC strains to three antibacterial agents was Am/CB/Te. The antibiograms of resistance to four antibacterial agents included Am/CB/S/Te, and C/CF/S/Te. Multiple resistance patterns of Shigella isolates are shown in Table 20. The patterns included Am/CB/CF, and Am/CB/Te. Similarly, resistance patterns of Staph. aureus are shown in Table 21. There were 19 different resistance patterns, and the most frequent were C/Cc/Dp/P/ Te/Va, E/Dp/P/S/Te/Va, C/Dp/p/S/Te/Va, and CF/Dp/k/p/S/Te.

Table 18. Single and Multiple Drug Resistance of Pathogens Isolated from the Different Samples.

Resistant to	Staph.aureus (19)		EPEC (26)		Shigella spp. (6)		Total (51)	
	No.	%	No.	%	No.	%	No.	%
One	2	10.5	2	7.6	0	0	4	7.8
Two	2	10.5	3	11.5	0	0	5	9.8
Three	6	32.6	1	3.8	1	16.7	8	15.7
Four	1	5.2	6	23.0	2	33.3	9	17.6
Five	0	0	6	23.0	0	0	6	11.8
Six	4	21.0	2	7.6	2	33.3	8	15.7
Seven	2	10.5	1	3.8	0	0	3	5.8
Eight	1	5.2	1	3.8	0	0	2	3.9
Nine	1	5.2	0	0	0	0	1	1.9
Total	19	100	22	84.6	5	83.3	46	90

Table 19. Antibiograms of Drug Resistance of Enteropathogenic *E. coli* Isolates

	Resistance antibiograms	No	%
	PB	1	3.8
	Te	1	3.8
	Am CF	1	3.8
	CF Te	1	3.8
	S SxT	1	3.8
Total EPEC	Am CB Te	2	7.7
test = 26	Am CB S Te	1	3.8
	Am CF S Te	2	7.7
	C CF S Te	1	3.8
	Am CB CF SXT Te	2	7.7
	Am C S SXT Te	1	3.8
	Am CB S SXT Te	1	3.8
	K PB S SXT Te	1	3.8
	Am C CB CF S Te	1	3.8
	Am CB CF S SXT Te	1	3.8
	Am C CB CF S SXT Te	1	3.8
	Am C CB K PB S SXT Te	1	3.8
	T o t a l	22	84.6

Table 20. Antibiograms of Drug Resistance of Shigella Isolates

	Resistance Antibiograms	No	%
Shigella strains tested = 6	Am CB CF	1	16.6
	Am C CB SXT	1	16.6
	Am CB Te	1	16.6
	Am CB CF S SXT	1	16.6
	Am CB CF S Te	1	16.6
	T o t a l	5	83

Table 21. Antibiograms of Drug Resistance of Staphylococcus aureus

	Resistance Antibiograms	No	%
Staph.aureus tested = 19	P	1	5.2
	Te	1	5.2
	P Te	1	5.2
	P Va	1	5.2
	C Dp Te	1	5.2
	C P Te	2	10.2
	Dp P Te	1	5.2
	E P Te	1	5.2
	K P Te	1	5.2
	CC E P Va	1	5.2
	C Dp P S Te Va	1	5.2
	CC CF Dp E P Va	1	5.2
	CF Dp K P S Te	1	5.2
	Dp E P S T Va	1	5.2
	CC CF DP E P S Va	1	5.2
	C' CC DP P S Te Va	1	5.2
	C CC CF DP E P Te	1	5.2
	C CF DP E GM K P S Te	1	5.2
	T O T A L	19	100

DISCUSSION

The present survey has attempted to examine the bacteriological quality of infant feeding bottles and feeds. The predominant bacterial isolates from samples of bottle- contents and teats were coliforms. Contamination of household utensils, foods, water etc., by the coliform group of bacteria has been reported from many countries. A study in rural kenya (Steenbergen et al., 1983) examined drinking water, and children's dishes, and the results have shown that 40 per cent of the dishes, and 2 per cent of the water were contaminated with these group of bacteria. Scotte et al.(1982) investigated microbiological contamination of 251 homes in Surrey, England. These investigators have isolated the coliforms such as E. coli, Citrobacter freundii, Klebsiella Pneumonia, and Enterobacter cloacae from toilet water, kitchen sink, dish cloth, cleaning cloth etc. The presence of the coliforms in food, or water samples suggests an objectionable bacteriological quality of such materials. This is because the presence of these organisms in food or water is evidence of the risk of contamination of these materials with potential pathogens, or potential spoilage, as well as sanitary conditions of food processing, production, or storage (WHO, 1987).

Because of the difficulty of isolating enteric pathogens from foods, most studies have used enumeration of coliform organisms as evidence of the potential for transmission of diarrhoeal

agents (WHO, 1989). Further more, some studies (Guarino et al., 1987; Arora et al., 1983) have incriminated some members of the coliforms in diarrhoeal etiology. Wadstrom et al. (1976) isolated Klebsiella, Enterobacter, Citrobacter, and other coliforms from Ethiopian infants with diarrhoea, and pointed out that these bacteria produce enterotoxins and could cause diarrhoea. Jiwa et al. (1981) isolated these coliforms from several foods and water samples in the same community. Earlier study on diarrhoeal etiology (Habte et al., 1964) analysed 49 feeding bottle samples from Addis Ababa, and the results showed that 15(31%) of these samples yielded coliforms. The coliform isolation rate (71%) in the present study is higher than the results obtained in the above study.

In the present study, enteropathogenic E. coli, Shigella, Staph. aureus, and Bacillus cereus have been detected from a small number of samples. Although the frequency of isolation of these enteric pathogens is relatively low, their very prevalence in both stool samples and infant foods, and feeding teats cannot be overlooked. A total yield of 9(3.7%) of EPEC from bottle-contents, in this study is comparable to the isolation rate of EPEC reported by Habte et al. (1964). The isolation rate of EPEC in the present study is by far lower than the rate reported from Zaria, Nigeria (Cherian et al., 1985; Cherian et al., 1987). These investigators have recovered a large group of bacteria including the coliforms from feeding bottle-contents; and EPEC constituted 29(58%) of their total bacterial isolates. A similar

study from Indonesia (Surjono et al., 1980) reported that E. coli was the predominant organism from bottle-contents, but none of the E. coli isolates in that study were the classically recognized pathogenic serotypes. In the present survey Staph. aureus was isolated as frequently as EPEC from the bottle-contents. The isolation ratos of Staph. aureus from elsewhere in Africa (Cherian et al., 1987, Cherian et al 1985) were higher than noted in this study. The isolation rate (16.4%) of Staph. aureus from 61 teat-swabs in this study, is almost five times it's isolation rate from samples of the bottle-contents. The isolation rate of Staph. aureus from feeding-teats in this study is comparable to 17 per cent recovery of Staph. aureus from feeding teats of Nigerian infants (Elegbe et al., 1982). The per cent recovery of Staph. aureus from feeding teats reported by Cherian et al. (1987) was slightly higher than the result obtained in the present study. Other investigators (Barrel et al., 1979; Black et al., 1982) used enumeration technique to determine Staph. aureus from food samples, hence it is not convenient to compare their results with the present finding. Isolation rate of Shigella Species from bottle-contents is very low in the present study. Literature survey on similar studies does not show a better recovery rate of Shigella from feeds and feeding utensils. For instance, no Shigella has been recorded in the reports from Nigeria, Uganda or Indonesia (Cherian et al., 1987; Philips et al., 1969, Surjono et al., 1980). No Salmonella organisms were detected in the present

study but reports from the Gambia (Barrel et al., 1979), and Nigeria (Cherian et al, 1985, and Elegbe et al., 1982) indicated the recovery of Salmonella from bottle-contents and teats.

The total bacterial counts observed in samples of most bottles in this study, agrees with reports from other studies (Surjono et al., 1980; Philip et al., 1969). In the present study, 59 per cent of all samples had bacterial count more than $1X10^6$ colonies of bacteria per ml. Although all types of bottle-contents showed heavy contamination, cereal blend or its mixture with cow's milk appeared to contain more frequently (65 per cent of all such samples) bacterial counts more than $1X10^6$ colonies per ml. The high bacterial counts observed in cereal preparation were also noted in the studies from the Gambia (Barrel et al., 1980), and elsewhere (Black et al.,1989). Barrel et altaph. (1980) suggested that the high bacterial count observed in cereal products could be due to poor heat penetration in such preparations. In the present study, cow's milk appears to be a major feed type given to babies in the studied population. Further more, it was found from the bacteriological analysis in this study, that cow's milk samples harboured a large number of bacteria including the enteric pathogens. The total bacterial counts of these samples were also among the highest. This could be due to the fact that milk provides a suitable culture medium for many micro-organisms. However, adequate boiling kills most of the bacteria in milk, particularly those in the family Enterobacteriaceae. Therefore, high microbial population

observed in this study indicate either inadequate boiling, or poor handling of the feeds after preparation. It may also be due to inadequate handling care of feeding bottles. In the present study high bacterial count as well as high rate of bacterial isolation have been observed in the rainy season. The rainy season in Ethiopia also coincides with increase in diarrhoeal cases (Stinzing et al., 1977). Barrel et al . (1979) observed a similar pattern in the Gambia. The explanation for high bacterial count and higher number of isolates in the rainy season may be, an increase in the unhygienic conditions in the environment such as frequent contamination of water.

The staus of mothers education in the present survey does not show a significant difference in the bacteriological quality of bottles and teats. Surjono et al . (1980) reported a similar observation from Indonesia. It is possible to learn from the answers given by mothers that feeds are prepared in bulk more than half a day in advance by the majority of mothers in the studied population. This type of practice has also been observed in other developing communties (Barrel et al ., 1979; Surjono et al., 1980; Roberts, 1982; Black et al., 1989). The vast majority of mothers in this study have indicated that they wash feeding bottles and teats only once or utmost twice, and most of these mothers have no extra bottles or teats (not shown in here). The frequency of bottle washing noted in this study is comparable to an observation in Nigeria (Cherian et al., 1985). Most of the

nursing mothers collect water from out door pipes a factor which may increase the chance of water contamination. The water can be even contaminated at home in the storage containers. In this study mothers have indicated that they usually rinse feeding bottles with unboiled water. This could have been an important source of pathogen recovery in bottle contents and teats. More over, about half of the nursing mothers indicated that they have maids or relatives to take care of the babies which normally includes feed preparation and feeding. Such house members could introduce pathogens into infant feeds as a result of faulty handling even when mothers are aware of the ill effect of poor handling of feeds and feeding bottles, and are careful.

Sixteen per cent isolation rate of enteric pathogens from fecal samples of the bottle-fed babies in the present study is comparable to the results from Kenya (Kalaya et al., 1972), and Nigeria (Akinterwa et al., 1982). The isolation rate of enteric pathogens in the present study, is lower compared to 37 per cent isolation indicated by Erwa et al. (1971) from the Sudanese children, and 42 per cent isolation rate from infants in South Africa (Housham et al., 1987). Previous investigators (Thoren et al., 1982) from Addis Ababa, Ethiopia reported a very high rate of pathogen isolation compared to the present finding. This was obviously due to the wide range of enteropathogens investigated by these authors. The isolation rate of enteric pathogens from diarrhoeal swab-cultures is slightly higher than the isolation of these pathogens from non-

diarrhoeal swab-cultures, but the difference was not statistically significant. Such a high yield of enteric pathogens from non-diarrhoeal healthy individuals has also been reported from India (Mathan et al., 1986). Enteropathogenic E. Coli was the predominant isolate from stools of both diarrhoeal and non-diarrhoeal babies. A similar observation was reported from the Sudanese Children (Erwa et al., 1971). Although EPEC was found in patients with gastrointestinal illness and those without gastroenteritis, the isolation rate of EPEC was significantly greater in the former group than in the latter. This finding agrees with reports from Uganda (Lubwama, 1986), and South Africa (Robin-Brown, 1980). Other investigators (Goldwater et al., 1981; Spencer et al., 1980; Sebodo et al., 1977) isolated EPEC in equal proportion both from patients and controls. The frequency of isolation in this study, agrees with the isolation frequency of EPEC reported previously from Addis Ababa (Thoren et al., 1982). In their report EPEC was listed as 2nd most frequent isolate next to rota virus. A similar work from Brazil (Coiro et al., 1987) listed EPEC as the 2nd most common isolate from Brazilian Children below 5 years of age. The isolation rate of EPEC from diarrhoeal stools in the present study is higher compared to 3.9 per cent, and 3.5 per cent reported by Suntadvoot et al., (1981), and Mahabir-Singh et al. (1988) respectively. The most frequent serotypes of EPEC detected in this study, have been also reported in previous study from Addis Ababa (Thoren et al., 1982). The serotypes of EPEC, 0126:K71 (B16), 0111:K58 (B4), and

O125:K63 (B15) were most frequent isolates in the present study. These serotypes of enteropathogenic E. Coli have also been reported from several studies (Antai et al., 1987; Lubwama, 1986; Edelman et al., 1983; Freij, 1973). The isolation of EPEC from sporadic cases of infantile enteritis has been unclear and controversial (Lubwama, 1986). However, epidemiological studies in developing countries (Lubwama, 1986; Antai et al., 1987; Robin-Brown, 1980; Gurwith, 1978) have demonstrated a significant association between EPEC and enteritis.

Shigella was isolated from 2(3.2%) of the 62 diarrhoeal stool samples. This result appears to be slightly higher compared to the isolation frequency of Shigella reported by Stintzing et al. (1977) from Ethiopian infants with diarrhoea. Similarly, the present result appeared to be higher from 4(1.6%) Shigella isolates out of two-hundred stool samples of children from Delhi, India (Mahbir-Singh et al., 1988). On the other hand, isolation rates of Shigella (8.3% and 9%) reported from Suth African infants (Hauseham et al., 1987; and Robin-Brown et al., 1985) were higher than the frequency of isolation of Shigella from Ethiopian infants and Children. Staphylococcus aureus was isolated from rectal-swab cultures of non-enteritis cases. Hone et al. (1985) have found fecal carriage of Staph aureus in infants with and withoutE. coli enteritis. The authors indicated that the carriage rate was significantly higher amongest infants with enteritis. The fecal strains of Staph aureus

may be acquired from the environment or from food (William cited in Hone et al., 1985).

Multiply resistant enteric pathogens are common in developing countries where indiscriminate use of antibiotics is a problem (Shears et al., 1987). The prevalence of resistant bacteria to commonly available antimicrobials in developing countries are contributing to the spread of major infectious diseases (Cheesbrough, 1984). It is not surprising that in the present study most of the bacterial isolates both from clinical samples, and feeding utensils were resistant to the commonly available antibacterial agents. The number of bacterial isolates tested in this study, were relatively low, however the results of the antimicrobial susceptibility test observed, are comparable to previous works (Obaseiki-Ebor et al., 1985, Shears et al., 1987). All the S. aureus strains isolated from stool and feeding teat samples in the present study were resistant to penicillin. Such a high rate of resistance to penicillin have been reported both from Ethiopia (Gedebou, 1982; Tewodros et al., 1983), and other country (Vymola et al ., 1987). The rate of resistance to penicillin observed in the present study is higher than what have been reported previously (Plorde et al., 1970). It has been clearly established that penicillin resistance of Staph. aureus strains is largely a result of beta-lactamase production (Frimodt-Moller et al ., 1986; Basker et al., 1980). In the present study, all penicillin resistant strains of Staph. aureus were

also found to be beta-lactamse positive. About 52 per cent of the Staph aureus isolates in this study have shown resistance to methicillin. Resistance to methicillin have been reported from Ethiopia (Plorde et al., 1970; and Gedebou, 1982), and else where (Hone et al., 1985). The percentage of methicillin resistant strains encountered in this study is higher than what have been indicated in the previous reports from Ethiopia. More than 70 per cent of the 19 Staph aureus strains in this study were resistant to tetracycline. This rate of resistance is by far higher than the resistance rate (19 per cent) reported by Plorde et al. (1970), but comparable to what have been reported by Gedebou (1982) from this country, and Vymola et al. (1987) from elsewhere.

Over 50 per cent of the enteropathogenic E. coli strains in this study were resistant to tetracycline and ampicillin. Like wise, sensitivity to carbenicillin and streptomycin was below 60 per cent. This observation agrees with reports from other countries (Shoars et al., 1987; Obaseiki-Ebor et al., 1985; Antai et al., 1987). Multiple resistance of EPEC seen in this study is also in agreement with what have been observed in the Sudan (Erwa et al., 1971), and in Nigeria (Obaseiki-Ebor et al., 1985). Five of the 6 Shigella isolates tested in the present study were resistant to three or more antibacterial drugs. Although the number of strains tested in the present study were very low, the resistance pattern of the Shigella strains to the commonly

available antibacterial drugs is comparable to previous records (Gebre-Yohannes et al., 1984; Gedebou et al., 1982). Such multiply resistant Shigella strains have also been reported from other part of the world (Panigrhai et al ., 1987). The most effective antibacterial agents in this study both against gram negative and gram positive bacteria were kanamycin, gentamicin, chloramphenicol, and trimethoprim-sulfamethoxazole, in that order. This observation is also comparable to several previous findings (Gedebou et al ., 1983; Gedebou, 1982; Piorde et al., 1970).

CONCLUDING REMARKS AND RECOMMENDATIONS

Despite the fact that this study has its own limitations, it can give a base line information for future studies on feeding practices. It appears that bottle-feeding is advancing in this community regardless of educational or occupational status of mothers. One important finding of this study is that mothers are aware of the ill effect of bottle-feeding and indicated their attempts to prepare sterile feeds for their babies. However, the feeding bottle-contents were invariably contaminated. There was no difference between the different educational levels of mothers in terms of bacteriological contamination of bottles. Preparation of feeds in advance of need combined with improper storage and inadequate cooking, and poor personal hygiene, could be factors that result in a highly contaminated bottle-contents.

In addition to fecal indicator organisms such as *E. coli* type I, multi-drug resistant enteric pathogens were isolated from bottle samples and stools of bottle-fed babies. This indicates that these organisms are circulating in the community, and bottle feeding serves as a vehicle in transmission of these pathogens. This study shows that infants and young children often ingest contaminated feeds whether they develop recurrent diarrhoea or not. This study can only measure what is happening at a single point in time. It is however reasonable to suppose that antecedent and successive feeds will be similarly contaminated

and the degree of contamination may vary from time to time. Similarly, the particular organisms present at a time may or may not be present to the child on another occassion. The isolation of enteric pathogens from stools of healthy and sick babies indicates that whether an infant suffers gastro enteritis or not depends not only on the fecal contamination of feeds, but also upon the child's ability to resist the establishment of the pathogens in his bowels.

From the present study it is possible that all types of bottle-contents may be potential vectors of pathogens in the studied community. However, despite the inherent dangers of bottle feeding, the number of bottle feeding mothers is continually increasing. Therefore the following recommendations may be forwarded:

1) In addition to its protective value, breast feeding is free of the potential hazards associated with artificial feeding such as contamination of feeding bottles, rubber teats, and cow's milk and other feeds. Hence, the supreme quality of breast milk should be constantly emphasized to mothers.

2) Further more, emphasis on proper handling of feeding utensils and feeds is essential: Bottle-feeding mothers should be taught to prepare infant foods for immediate consumption only, and to reheat feeds that have been

stored for a long time, to a proper temperature for sufficient time. The teaching of mothers who use bottle feeding must be accompained by regular home visit to observe both feeding utensils and home environment.

3) The indiscriminate use of anti-bacterial drugs must be controlled to avoid the spread of drug resistant enteric pathogens.

4) Lastly, well controlled community based longitudinal studies are needed on:-

   A. the association between diarrhoeal disease and isolation of enterotoxigenic coliform bacteria.

   B. the point and source of contamination of infant foods.

   C. developing effective home made sterility procedures.

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APPENDIX

14. How often do you wash the feeding - bottles and teats ?

A) Once daily B) twice daily C) Three times daily

D) After every feed.

15. How many feeding - bottles do you have ?

A) No extra bottle B) 2 C) 3 D) More than three

16. Why did the child come to the hospital ?

_____

17. Did the child have any symptom of diarrhoea or abdominal problem during the last 10 days ?_____

18. If maid is available; A) What is her role ?_____

_____

19. Clinical diagnosis _____ _____

(information to be obtained from the physician).