ISOLA TION OF ENTERIC PATHOGENS AND COLIFORM BACTERIA FROM INFANT FEEDING BOTTLE CONTENT IN ADDIS ABABA, ETHOIPIA

Zeleke wrrensay*,BSc,MSc, & Haile Selassie Tesfaye,BSc, MSc, PhD**

ABSTRACT: Gastro-enteritis causing bacterial pathogens were studied in infant feeding bottle-contents collected from 244 feeding bottles which were brought to 5 clinics and 2 hospitals from January 1989 to November 1989 in Addis Ababa, Ethiopia.

The most frequent bacterial isolates were coliform which included Enterobacter spp. ,Klebsiella spp., faecal E.coli and Cotrobacter spp. Enteric pathogens like enteropathogenic E. coli (EPEC), Shigella spp., and Staph. aureus, constituted respectively (3.3%), 1 (0.01 %),9 (2.2%) of the total isolates.

Although the percent of isolation of enteric pathogens in this study is low, the frequent isolation of Coliform from samples of bottle-contents suggests that the bottle-feeding serves as a vehicle in transmission of the enteric pathogens in the studied population.

Bottle-feeding mothers should be constantly taught on proper handling of feedingutensils and feeds. Above all the superior quality of breast milk needs to be emphasized to nursing mothers. Moreover, further and well controlled studies are also recommended to reduce diarrhoeal diseases in infant and young children.

INTRODUCTION

Many studies (1,2,3) have demonstrated an association between infant feeding practice and infant health. The majority of these works indicated that infant morbidity and mortality are influenced by the mode of infant feeding practice.

Artificial feeding of infants is a method which for success relies upon maintenance of high degree of hygiene in the home. In developed countries with good sanitation, nutrition and medical care, bottle-feeding is less risky than in the set up of the developing countries (2,4). Since the standard of personal hygiene and public sanitation is low in many communities of developing nations, contamination of infant feeds with pathogenic micro-organisms may be an important source of infectious diarrhoea (5). Bacteriological survey of feeds and feeding bottles from Africa and other countries (6,7) have shown gross contamination of feeding utensils and feeds.

In Ethiopia, gastro-enteritis has been a major disease problem among infants and young children (8,9,10). Studies from Ethiopia (II), South Africa (12) and from other countries (13) on paediatrics diarrhoea indicate that enterotoxigenic coliform such as Klebsiella, Entero-bacter, and Citrobacter are putative casual organisms in addition to known enteric pathogens. Although gastro-enteritis is a major cause of morbidity and mortality in infants and young children, bacteriological studies of infant food and feeding utensils and its influence on the health of bottle-fed babies in Ethiopia are very scarce. Therefore, the aim of the present study is to investigate the importance of infant feeding bottle-contents as a vehicle of bacterial enteric pathogens in Addis Ababa, Ethiopia. The specitic objective of the study is to isolate and identify enteric pathogens. The study does not attempt to isolate pathogens which have special isolation requirements such as Campylo bacter jejuni, Yersinia enterocolitica, or diarhoeagenic E. coli other than Enteropathogenic E. coli (EPEC).

*Jimma Institute of Health Science, Jimma, Ethiopia
**National Research Institute of Health, Addis Ababa, Ethiopia

MATERIAIS AND METHODS

Feeding bottle-contents 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 babies had 'varying complaints such as fever, cough and diarrhoea, while some were brought for clinical checkup and routine immunization.

After thorough shaking, about 10ml of the bottle-contents were transferred into sterile screw-capped container. The samples were transported to the bacteriology laboratory in the National Research Institute of Health, Addis Ababa, within 1-2 hours of time.

In the laboratory the bottle-contents were inoculated on to Mac-Conkey, Salmonella Shigella, Mannitol Salt and Sheep Blood Agar prepared from Difco Powders. The cultured plates were then incubated for 24-48 hours at 37°C aerobically. Furthermore, 2.5ml volume of the bottle-contents were removed from the original samples with sterile pipettes and passed into lactose broth as suggested by Isom (18) for pre-enrichment of Salmonella species. After an over night incubation at 3^0C, 2.5ml volume of the pre-enriched culture were transferred into Tetrathionate enrichment broth.

Tryptone soya yeast (TSY) enrichment broth was used for enrichment of other pathogens. The broth cultures were incubated at 37°C for 48-72 hours, after which inocula were taken with sterile pasteur pipettes and inoculated into Mac-Conkey, Salmonella-Shigella, Mannitol Salt and Blood Agars. Bacterial colonies on Mac-Conkey and/or Salmonella-Shigella agars were differentiated on the bases of fermentation reactions as
lactose fermenting or non-lactose fermenting colonies. The standard biochemical techniques suggested by Cheesbrough (19) were used for identification of Salmonella, Shigella and other Enterobacteriacae. Salmonella, Shigella species, and entero-pathogenic E. coli (EPEC) were further confirmed serologically with respective antisera for these organisms, obtained from Difco laboratories. Gram positive bacteria such
as Staphylococcus species and Bacillus species were looked for, on blood and mannitol salt agars and identified with combination of Gram stain, Catalase and Coagulase test, and whenever necessary by using appropriate biochemical tests described by Cowan and Steel (20).

RESULTS

Table I shows the types of bottle-contents (feeds) analyzed. Table 2 shows the various bacterial species isolated from different bottle-contents. As can be seen from Table 2, 270 bacterial isolates were recovered from a total of 244 samples of bottle-contents. A total of 26 bacterial strains were recovered from 17 samples
of cereal blends. Fresh cow's milk samples yielded 165 different bacterial isolates. 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 isolates, 9 (14.2%) were the classically recognized serotype of entero-pathogenic E. coli
(EPEC). Shigella species was isolated from only one sample.

Table 1. Type of bottle-contents (feeds) analyzed Bottle-content (feeds) analyzed

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

Staphylococcus aureus consisted 9 (3.3%) of the total bacterial isolates. Of the 9, Staph. aureus isolate 5 (55.5%) were detected from fresh cow's milk. No Salmonella or Vibrio species were isolated from any of the bottle- contents. The predominant isolates were the coliform bacteria which included Enterobacter spp. 66 (22.4%), Klebsiella spp. 50 (18.55%), and Citrobacterspp. 31 (11.5%).

Table 2. Bacterial isolates fraIl different samples of bottle-contents.

Bacterial isolates	Fresh cow's milk (151)		Commercial milk (33)		Cereal (17)		Cow's milk + cereal (28)		Others (15)		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Enteropathogenic E.coil	5	3.0	4	11.4	0	0	0	0	0	0	9	3.3
E.coil type I	30	18.8	4	11.4	5	19.2	5	17.8	1	6.3	45	16.7
Other E.coil...	5	3.0	0	0	1	3.8	3	10.7	0	0	9	3.3
Shigella flexneri	1	0.01	0	0	0	0	0	0	0	0	1	<0.01
Staphyloccous aureus	5	3.0	1	2.8	1	3.8	1	3.6	1	6.3	9	3.3
Bacillus spp.	6	3.6	0	0	0	0	0	0	0	0	6	2.2
Enterobacter spp.	29	17.6	12	34.2	11	42.3	7	25.0	7	43.0	66	24.2
Citrobacter spp.	25	15.5	1	2.8	2	7.6	3	10.7	0	0	31	11.5
Klebisella spp.	35	21.1	6	17.1	1	3.8	6	21.4	2	12.5	50	18.5
Proteus spp.	2	1.2	0	0	0	0	0	1	1	6.3	3	1.1
Acineto bacter spp.	12	7.2	3	8.5	1	3.8	1	3.5	1	6.3	18	6.6
Pseudomonas spp.	2	1.2	2	5.7	0	0	0	0	0	0	4	1.4
Streptococcus spp.	4	2.4	1	2.8	2	7.6	1	7.1	0	0	9	3.3

Other organisms(Yeasts, unidentified spp.)	4	2.4	1	2.8	2	7.6	0	0	3	18.8	10	3.7
Total	165	100	35	100	26	100	28	100	16	100	270	100

NB. . Powder milk formula milk or formula, "Tea, water, Oral rehydration solution etc.,
... Biochemically conform to E.Coil but serologically different from EPEC.

DISCUSSION

The present study has some limitations such as inability to test toxigenicity of S. aureus and the coliform bacteria. Despite these limitations the study has attempted to investigate the bacteriological contamination of infant feeding bottle contents. The predominant bacterial isolates from samples of bottle-contents were coliform such as Enterobacter spp., Klebsiella spp., Citrobacter spp. and E. coli. Contamination of household utensils, foods, water, etc. by coliform group of bacteria has been reported from many countries (14,15). These bacteria have also been reported previously from Ethiopian infants with diarrhoea (11) and from food and water (17). Although the present study has not shown that the coliform organisms are toxigenic, previous studies (11, 13) demonstrated that some of these bacteria are toxicogenic and could cause diarrhoeal disease in infants and young children. Earlier study on diarrhoeal etiology (16) analyzed 49 feeding bottle samples
from Addis Ababa and the results showed that 15 (13%) of their samples yielded coliform.

In the present study enteric pathogens such as entropathogenic E. coli (EPEC), Shigella spp. and S. aureus have been detected from a small number of samples. The total yields of9 (3.3%) EPEC from bottle-contents in this study is comparable to the isolation rate reported by Habte et aI (16). The frequency of isolation of
EPEC in the present work is by far lower than the rate reported from Zaria, Nigeria (2). EPEC constituted 29 (58%) of the isolates in the above report. The recovery of S. aureus in the present study is comparable to what was reported from Zaria, Niger in a similar study (2). Isolation rate of Shigella spp. from bottle-contents in the present study is very low. Literature review on a similar studies does not show a better recovery rate of Shigella from feeds and feeding utensils. For instance, no Shigella spp. has been recorded in the reports from Nigeria (2), Uganda (21) and Indonesia (7).

All different types of bottle-contents in this study were found to be contaminated with coliform bacteria. However, there are slight difference in tile degree of contamination. This observation agrees with what have been noted by workers in other co\mtries (5, 22).

CONCLUSION AND RECOMMENDATION

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. The result of the study shows that all classes of bottle contents (bottle feeds) are potential vectors of enteric pathogens. Hence infants and young children in the studied population often ingest contaminated feeds whether they develop recurrent diarrhoea or not.

Breast milk is free of potential hazards associated with bottle-feeding and superior in its protective value (16). Therefore, breast-feeding should be constantly emphasized to mothers. On the other hand, bottle-feeding mothers should be taught on proper handling of feeding utensils and feeds. The teaching of mothers who practice bottle feeding must be accompanied by regular home visit to observe both feeding utensils and
home environment. Furthermore, well controlled community based longitudinal studies are needed if we are to reduce gastro-enteritis in babies as the result of using feeding bottles.

REFERENCES

1. Jelliffe, D.B. and Jelliffe E.F. Feeding utensils and infant diarrhoea in less developed countries. Am. J. Vis. a.ild. 1982; 136: 167.
2. Cherian, A. and Lawande R.V. Recovery of potential pathogens from feeding bottle-contents and teats in
Zaria, Nigeria. Trans. R. Soc. Trap. Med. Hyg. 1985; 79: 840-842.
3. Anderson, J.A.D. and Gatherer, A. Hygiene of infant feeding utensils, practice and stsnd.rd in the home. Br. Med. J. 1970; 2: 20-23.
4. Kovar, M.F. Serdela, M.K., MarksJ.S., FraBer, D.W. Review of the epidemiologic evidence for an association between infant feeding and infant health. Pediatrics. 1984; 74: 615.
5. Black, R.E., Romano, G.L., Brown, K.H., Bravo, N., Bazalar, O.G., and Kanoshiro, H.C. Incidence and
etiology of infantile diarrhoea and major routes of transmission in Huscar, Peru. Am. J. Epidemial. 1989;
129: 785-799. 6. Drejer, G.F. Bottle feeding in Doulala, Cameroons. J. Trap. Pedialr. 1980; 26: 24-35.
7. Surjone, D., Ismadi, S.D., Suwardji and Rhode, J. Bacterial contamination and dilution of milk in infant feeding bottles. I. Trop. Pedialr. 1980; 26: 58-61.
8. Frij, L. Observation on parasitic and bacterial infections of the intestine among paediatric out-patients in Addis Ababa, Ethiopia. Eth. Med. I. 1973; II: 13-24.
9. Stintzing, G., Tufvesson, B. Habte, D., Back, E., Johnson, T . and Wadstrom, T. Etiology of acute diarrhoeal disease in infancy and childhood during the peak seasons, in Addis Ababa. A preliminary report. Eth. Med. I. 1977; 15: 141-146.
10. Thoren, A., Stintzing, G., Tufvesson, B., Walder, M. and Habte, D. Etiology and clinical features of severe infantile diarrhoea in Addis Ababa. Eth. Med. I. Trop. 1982; 28: 127-131.
11. Wadstrom, T., Aust-Kettis, A.S., Habte, D., Holmgren, J.,Meevwisse, G. Enterotoxin producing bacteria and parasites in stools of Ethiopian children. Arch. Dis. a.ild. 1976; 15: 865-870.
12. Schub, B.D., Greeff, A.S., Lecatsas, G., Prozesky, O.O.W., Hay, J.T., Prinsloo, J.G. and Ballard, R.C. A
Microbiological investigation of acute summer gastroenteritis in black South African infants. I. Hyg. Cam-
bridge. 1977; 78: 377-385.
13. Guarino, A., Campano, G., Malamisura, B., Alessio, M., Guandalin and Rubino, A. Production of Escherichia coli Sts -like Heat Stable entertoxin by Cilrobacler freundii isolated from humans. I. Clin. Microbiol. 1987; 25: 110-114.

14. Steenbergh, W.U., Mossel, D.A., Akusia, J.A. and Jansen, A.A.J. Machakos project studies: Agents affecting health of mother and child in a rural area of Kenya. Trop. Geog. Med. 1983; 35: 193-197.
15. Pickering, H. Social and environmental factors associated with diarrhoea and growth in young children: Child health in urban Africa. Soc. Sci. Med. 1985;21: 121-127.
16. Habte, D. and Debesai A. Etiology of infantile gastro-enteritis. Eth. Med. I. 1964; 3: 191-24.
17. Jiwa, S.F.H., Krovcek, K. and Wadstrom, T. Entero-toxigenic bacteria in food and water from an Ethiopian community. App. Environ. Microbiol. 1981; 41: 1010-1019.
18. lsom, B.G. Compedium of methods for the microbiolo2icalexamination of foods. Ist. ed. American Public Health Association, Washington D.C. 1976.
19. Cheesbrough, M. Medical laboratorv manual for Tropical countries II, Microbiology. Ist ed. F.L.M.L.S. Tech. R.M.S. 1984.
20. Cowan, S.T. and Steel, K.J. Manual for the identification of medical Bactoria. 2nd ed. Cambridge University Press, Cambridge.1974.
21. Philip, 1., Lwanga, S.K., Lore, W. and Wasawa, D. Methods and hygiene of infant feeding in urban area of Uganda. I. Trop. Pedialr. 1969; 15: 167-171.
22. Hibbert, J.M. and Gorden, M.H.N. What is the weanling dilemma? Dietsry faecal Bacterial ingestion of normal children in Jamaica. I. 1i-op. Padialr. 1981; 27: 225-258.