

Enterotoxin-producing bacteria and parasites in stools of Ethiopian children with diarrhoeal disease

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Wadström, T., Aust-Kettis, A., Habte, D., Holmgren, J., Meeuwisse, G., Möllby, R., and Söderlind, O. (1976). *Archives of Disease in Childhood*, 51, 865. **Enterotoxin-producing bacteria and parasites in stools of Ethiopian children with diarrhoeal disease.** Enterotoxinogenic bacteria were isolated from 131 (37%) of 354 Ethiopian infants and children with acute gastrointestinal symptoms. Only one of these isolates belonged to the classical enteropathogenic serotypes of *Esch. coli*. Two colonies from each patient were isolated and tested for production of enterotoxin by the rabbit ileal loop test, the rabbit skin test, and an adrenal cell assay. However, only 38% of the isolated enterotoxinogenic strains were *Esch. coli*; the others belonged to *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter*, *Serratia*, and *Aeromonas*. In 18 patients both isolates were toxinogenic and belonged to different species. The incidence of intestinal parasites was 35% with no apparent correlation to the occurrence of toxinogenic bacteria in the stools.

Diarrhoeal disease of early childhood is common all over the world and the prevalence is less influenced by climate than by other environmental factors (Gordon, 1971). In the developing countries like India and Guatemala high incidences of diarrhoeal disease have been reported with death rates among infants of 17/1000 and in children 1 to 4 years old of 21/1000, which is 519 times the rate in the United States (Gordon, 1971; Mata and Urrutia 1971). Many micro-organisms have been associated with acute diarrhoea but established pathogens can be isolated in only less than half the patients in epidemiological studies both in industrialized and nonindustrialized countries (Freij, 1973; Drachman, 1974).

Vibrio cholerae causes diarrhoea by producing an enterotoxin (Formal, DuPont, and Hornick, 1973). Recent studies have shown that other Gram-negative enteric bacteria can also cause diarrhoea by enterotoxin. Enterotoxinogenic *Esch. coli* was first found to cause diarrhoea in piglets and calves, but more recently in children and adults as well in India and Vietnam (Gorbach and Khurana, 1972; DuPont

et al., 1971). However, few epidemiological studies have been made on the relative importance of enterotoxinogenic bacteria compared to conventional pathogens in acute diarrhoeal disease.

Enteritis and intestinal parasitic infection constitute a major health problem in Ethiopian children (Freij, 1973). Among new outpatients of the Ethio-Swedish Pediatric Clinic in 1973, 12% suffered from enteritis. No established viral, bacterial, or parasitic intestinal pathogen was found in 55% of the cases, and enteropathogenic *Esch. coli* serotypes were isolated in only 10% of the cases (Freij, 1973).

The aim of our study was to assess the relative frequency of enterotoxinogenic bacteria, irrespective of species, among Ethiopian children with diarrhoea or other gastrointestinal complaints. An attempt was also made to determine whether a correlation existed between the coincidence of enterotoxinogenic bacterial strain and intestinal parasites.

Material and methods

Patients. Stool specimens were randomly sampled from 386 individuals attending the outpatient depart-

ment of the ESPC and the Kirkos Health Clinic in Addis Ababa, which cater mainly for children of low socioeconomic groups. Specimens were collected from consecutively registered patients, regardless of history of gastrointestinal disease, in an attempt to gather unbiased information. The following criteria were applied before including patients in the study. None was receiving antibiotics or chemotherapy, or was on treatment for any other disease, but parenteral rehydration for up to 3 days before the sampling was acceptable.

The peak incidence of intestinal infections occurs during February-May. Our sampling was done during April-September 1974. There were no epidemics in Addis Ababa at the time, nor was the urban population markedly affected by the drought and famine which struck other parts of Ethiopia at that time.

The 354 patients were grouped according to age and symptoms as follows. Age group (I) 0-12 months, (2) 1-4 years, (3) >4 years (Jelliffe, 1966). Symptom groups (I) watery (or) 'cholera-like' diarrhoea, i.e. voluminous watery stools dehydrating the patient, often causing great difficulty in recovering faecal material in the liquid masses, (II) diarrhoea with blood and/or mucus (or 'dysentery-like') with more than 4 stools over 24 hours. The diarrhoeal stools were loose, foul smelling, and contained macroscopical blood and/or mucus. (III) Gastrointestinal symptoms with no diarrhoea but more than one of the following symptoms: nausea, vomiting, colicky pains, flatulence, and abdominal distension. A fourth group comprised only 17 children without any gastrointestinal complaints and with normal stool findings. This group, however, was not considered representative as a control group, and was not included in this report. In addition, samples were taken from 15 healthy student nurses at the clinics.

Bacteriological methods. Stool specimens were inoculated in the Stuart transport medium (Kallings, 1968) and flown to Stockholm within 10 days without any special cooling precautions. Isolation and primary identification were carried out according to Edwards and Ewing (1972). Each stool specimen was plated on horse-blood, deoxycholate citrate (DC), and Endo and thiosulphate-citrate-bile salts sucrose (TCBS) agar plates. Kauffman tubes were inoculated and incubated overnight before the organisms were plated on additional DC and Endo agar plates. Two colonies with different morphology on Endo agar plates were subcultured on Endo agar and stored on deep agar tubes at 20 °C for subsequent biochemical tests and serological typing. All strains thus isolated were inoculated in brain heart infusion broth (BHI, Difco) in tubes containing 10 ml of liquid medium. The tubes were incubated on a shaker (200 rpm, 16 h, 37°) and centrifuged (4000 × g × 20 min, 4°). Supernatants were then tested for enterotoxin activity.

Biochemical typing was performed with the API system (Analytab, New York, N.Y.) as recently described (Nord, Wadström, and Dahlbäck, 1976). Each toxigenic isolate was also identified in a second laboratory

independently, using the methods of Edwards and Ewing (1972). Each isolate of *Esch. coli* was agglutinated with a polyvalent serum for enteropathogenic serotypes of *Esch coli*; *Salmonella* and *Shigella* were identified with standard methods using antisera prepared at the National Bacteriological Laboratory. O-serotyping of enterotoxinogenic strains was done by Drs. Ida and Frits Ørskov at the WHO Escherichia Reference Centre, Copenhagen.

Tests for enterotoxin. Enterotoxin activity was tested by three methods: (1) rabbit intestinal loop test, (2) rabbit skin test, and (3) adrenal cell test. Undiluted 1 ml culture supernatants were injected in jejunal loops in young adult New Zealand rabbits starting approximately 50 cm from the pylorus (25 loops per rabbit) (Holmgren, Söderlind, and Wadström, 1973). The skin test was performed as recently described with 0.1 ml undiluted samples (Pierce and Wallace, 1972). The adrenal cell assay was performed twice in 1/10 dilution of each specimen (Donta *et al.*, 1974). All three tests were performed in duplicate by the same person unaware of the history of the patient.

Strains classified as toxigenic fulfilled the following criteria. (1) The results of the adrenal cell test were positive twice. (2) They gave positive rabbit intestinal loop tests and/or rabbit skin tests. All assays were performed on supernatants stored undiluted at -20° for less than one week after cultivation.

Parasitological methods. All stool specimens obtained were examined fresh for ova and protozoa and again after formolether concentration according to Ridley and Hawgood (1956).

Results

Table I shows the distribution of 354 patients in the age and symptoms groups. The youngest age group was predominate in symptom group I with watery diarrhoea, while in group II children 1 to 4 years of age predominated. This group contained 38% (135) of the patients.

TABLE I
Distribution of the 354 Ethiopian outpatients, grouped according to age and symptoms

Age groups	Symptom groups*			Total
	I n (%)	II n (%)	III n (%)	
(1) 0-12 m	47 (13)	61 (17)	0	108 (31)
(2) 1-4 yr	17 (5)	135 (38)	7 (2)	159 (45)
(3) >4 yr	0	49 (14)	38 (11)	87 (25)
Total	64 (18)	254 (69)	45 (13)	354 (100)

*I, watery diarrhoea; II, bloody and/or mucus diarrhoea; III, gastrointestinal symptoms; no diarrhoea.

Two bacterial isolates from each stool culture were assayed in the rabbit intestinal loop test, rabbit skin test, and the adrenal cell test before the species of each strain was diagnosed by biochemical tests. The frequency of enterotoxinogenic (ent⁺) strains in the different age and symptom groups is listed in Table II. A remarkable finding was the high

(5.1%) of the patients, where both isolates were ent⁺, the strains belonged to different bacterial species.

In the whole material 24% (173/710 strains) were ent⁺, while 27% (66/244) of all *Esch. coli* strains isolated were ent⁺. Thus 23% (107/466) of all strains belonging to species other than *Esch. coli* were found to be enterotoxinogenic. All strains of *Esch. coli* were O-grouped, and only one single ent⁺ strain was found to belong to the classical enteropathogenic serotypes; of the ent⁻ strains of *Esch. coli* (178 strains) only 6 strains were of enteropathogenic serotypes. They were equally distributed over the different symptom and age groups.

TABLE II

Distribution of patients with enterotoxinogenic bacterial isolates by age and symptom groups

Age groups†	Symptoms†			Total
	I n (%)	II (n %)	III n (%)	
1	19 (40)*	21 (34)	—	40 (37)
2	5 (29)	49 (36)	2 (29)	56 (35)
3	—	22 (45)	13 (34)	35 (40)
	24 (38)	92 (38)	15 (33)	131 (37)

*Number of patients with enterotoxinogenic strains and in parentheses the percentage of the total number of patients in the respective group (according to Table I).

†See Table I.

incidence of ent⁺ strains in all groups, 37% (131), with no significant difference between the age and symptom groups. The stools of 4 of the 15 student nurses also contained ent⁺ strains, which might be explained by close contact with children with ent⁺ diarrhoea.

Biochemical testing in two different laboratories with a conventional biochemical test and with a commercial kit (API) gave the same diagnosis of species in all but three of the 710 strains from the 369 individuals. An interesting finding was the relatively high frequency of ent⁺ enteric bacteria other than *Esch. coli* (Table III). Only 11 strains of *Salmonella* and 8 of *Shigella* were found in the whole material, all of them nontoxigenic. In 18

TABLE III

Species of enterotoxinogenic bacterial strains isolated

Species	Strains	%
<i>Esch. coli</i>	66	38
<i>Klebsiella</i>	26	15
<i>Enterobacter</i>	21	12
<i>Citrobacter</i>	19	11
<i>Proteus</i>	12	7
<i>Aeromonas</i>	11	6
<i>Serratia</i>	4	2
<i>Pseudomonas</i>	1	1
Not typed	13	8
Total	173	100

Recovery of parasites. The results of the parasitological stool examination in the three different symptom groups are summarized in Tables IV and V. In group I with watery, 'cholera-like' diarrhoea the incidence of parasites was low. This

TABLE IV

Distribution of patients with parasites in their stools by age and symptom groups

Age groups	Symptoms			Total
	I n (%)	II n (%)	III n (%)	
1	2 (4)*	8 (13)	—	10 (9)
2	1 (6)	63 (47)	1 (14)	65 (41)
3	—	26 (53)	22 (58)	48 (55)
Total	3 (5)	97 (40)	23 (51)	123 (35)

*Number of patients with parasites and in parentheses the percentage of the total number of patients (354) in the respective group (according to Table I).

TABLE V

Distribution of patients with parasites and enterotoxinogenic bacterial isolates by age and symptom groups

Age groups	Symptoms			Total
	I n (%)	II n (%)	III n (%)	
1	1 (5)*	3 (14)	—	4 (10)
2	1 (20)	22 (45)	1 (50)	24 (43)
3	—	10 (45)	6 (46)	16 (46)
Total	2 (8)	35 (38)	7 (47)	44 (34)

*Number of patients with parasites among those with enterotoxinogenic bacterial isolates in the respective group (according to Table II); percentage in parentheses.

could be due not only to the difficulty in finding faecal material in the voluminous liquid masses, but also to the fact that these patients were predominantly younger than one year, at which time there is normally a lower rate of infestation. It is obvious from Tables IV and V that there was no correlation between the incidence of parasites and ent⁺ strains.

Discussion

Most of the children (45%) with diarrhoeal disease belonged to the age group 1 to 4 years (group 2, Tables I and II), which agrees with previous studies from developing countries (Gordon, 1971; Mata and Urrutia, 1971; Drachman, 1974). The children came mainly from low socioeconomic groups, and many case histories could be correlated with poor sanitation. Those with cholera-like disease (group I, Table I) had an acute onset and were usually in bad condition. The children with dysentery-like disease (group II, Table I) were on average older.

The parasitological findings agree with those in a study from Botswana (Cooper and Johnson, 1973) but contrary to that study we, and Freij (1973), found only a few enteropathogenic *Esch. coli* strains. *Shigella* and *Salmonella* were also isolated only from a few cases. This may in part be due to the long transport.

It is of interest that the incidence of the enterotoxinogenic strains was similar in the different age and symptom groups, i.e. no predominance of such strains was noted in the cases with watery, cholera-like diarrhoea. Together with reports from other geographical areas the present findings suggest a world-wide high incidence of enterotoxinogenic bacteria in diarrhoea of children as well as adults (Gordon, 1971; Drachman, 1974; Formal *et al.*, 1973; Gorbach and Khurana, 1972).

An altered distribution of *Esch. coli* in the mouse intestinal tract was recently noted after oral infection with a metazoan pathogen (Cypess *et al.*, 1974). Since disease with enterotoxinogenic and enteropathogenic *Esch. coli* is dependent on colonization of the upper small intestine both in animals and children (Challacombe *et al.*, 1974; Heyworth and Brown, 1975) this suggests that parasitic infections may play a role in the pathogenesis of bacterial infantile diarrhoea (Freij, 1973; Drachman, 1974). We did not, however, find any correlation between the incidence of parasites in faecal and enterotoxinogenic stool isolates.

The most common parasite isolated in our study was *Ascaris* (12%), followed by *Entamoeba histolytica* (9%). *Giardia lamblia* was recovered in 5%. A greater number of parasites was diagnosed

with increasing age, and an overall incidence of *E. histolytica* (10–12%) was found to agree with a previous study from the same clinic (Freij, 1973). A high incidence of ova was found in all age groups without any apparent correlation with the clinical symptoms.

A recent study on travellers' diarrhoea in Mexico (Gorbach *et al.*, 1975) showed a high incidence of toxinogenic isolates (68%). In a study from a nursery in Brazil 85% harboured enterotoxinogenic strains in their stools during the period of acute diarrhoea (Guerrant *et al.*, 1975). In a search for enterotoxinogenic *Esch. coli* in 59 Apache Indian children only 16% of the episodes of diarrhoea were found to be caused by such organisms (Sack *et al.*, 1975). However, since biochemical identification of species and serotyping of *Esch. coli* probably preceded the bioassays in these studies, the incidence of enterotoxinogenic enteropathies (Formal *et al.*, 1973) might have been higher when compared to this study.

The overall high incidence of enterotoxinogenic strains of *Esch. coli* and other Gram-negative enteric organisms such as *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter*, and *Yersinia* sp. (Acres *et al.*, 1975; Koupal and Deibel, 1975) and also of *Aeromonas* and *Pseudomonas*, which are taxonomically related to *V. cholerae* (Staley and Colwell, 1973), is an interesting epidemiological finding. Klipstein *et al.* (1973) recently reported on toxinogenic *Klebsiella* and *Enterobacter* isolated from jejunal aspirates of patients with tropical sprue. In a recent study on acute diarrhoea in calves an isolate of *Citrobacter* gave a positive intestinal loop test (Acres *et al.*, 1975). It is thus possible that either a whole family of enterotoxins may exist in different Gram-negative species, even in such pathogens as certain *Salmonella* and *Shigella* sp., or that plasmids controlling enterotoxin production can spread between related species as can antibiotic resistance factors (Gyles, So, and Falkow, 1974; Lacey, 1975).

The high incidence of toxinogenic strains of species other than *Esch. coli* (Table III), previously unreported, might be explained by the fact that in our study colonies were tested for enterotoxigenicity before the final biochemical typing to species level. Plasmid control of production, at least of *Esch. coli* enterotoxin (Gyles *et al.*, 1974), and the fact that there is no correlation of toxin production with the classical enteropathogenic serotypes as described by Gorbach and Khurana (1972) were confirmed in this study. This shows the importance of bioassays for diagnostic and epidemiological studies of acute diarrhoeal disease in children, adults, and animals. The high incidence of enterotoxinogenic isolates

reported in this study (Table II) is a minimum value since overgrowth of certain strains and suppression of others in the Stuart transport medium cannot be excluded.

The rabbit intestinal loop assay is the classical test both for cholera and *Esch. coli* enterotoxin. However, this is probably not sensitive enough for detection of toxin in broth cultures for all enterotoxinogenic strains (Gorbach and Khurana, 1972; Klipstein *et al.*, 1973). A tissue culture system for toxin detection is 100 times more sensitive and is simpler to use when a large number of strains are investigated as in this study.

The adrenal cell model and tissue culture systems like Chinese hamster ovary cells (Guerrant *et al.*, 1975) measure the heat labile toxin, and since strains might exist which only produce heat-stable toxin (Gorbach, 1974), the intestinal loop assay which detects both types of toxin should probably not be excluded from further epidemiological studies. This comparative study confirmed that the cell test was more sensitive, simpler to perform, and less time-consuming than the others.

Some strains were difficult to assay in all three test systems, since they produce other toxins such as haemolysin and probably other cytotoxins as well. There were test samples that gave dermonecrosis in the skin test instead of the typical blueing of cholera and *Esch. coli* enterotoxin. Some of these strains might have been enterotoxinogenic but had to be excluded since they did not fulfill the criteria of this study. However, comparisons with each strain in different laboratories gave good agreement among the three tests. The skin test was more difficult to read and interpret than the other two, however.

Epidemiological studies on diarrhoeal disorders in different geographical areas will extend our knowledge of this global health problem. Together with various toxinogenic enterobacteria, viruses such as rotavirus may account for most of the undiagnosed cases of acute diarrhoea (Davidson *et al.*, 1975). Furthermore, *Esch. coli*, and perhaps related species also, is known to cause diarrhoeal disease by invading the intestinal mucosa without producing an enterotoxin (Formal *et al.*, 1973; DuPont *et al.*, 1971). When suitable laboratory methods have been developed for detecting such invasive strains as well, the days when no aetiological agent could be found in most patients with acute diarrhoea will be past.

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REFERENCES

- Acres, D. S., Laing, C. J., Saunders, J. R., and Radostits, O. M. (1975). Acute undifferentiated neonatal diarrhoea in beef calves. 1. Occurrence and distribution of infectious agents. *Canadian Journal of Comparative Medicine and Veterinary Science*, **39**, 116.
- Challacombe, D. N., Richardson, J. N., Rowe, B., and Anderson, C. M. (1974). Bacterial microflora of the upper gastrointestinal tract in infants with protracted diarrhoea. *Archives of Disease in Childhood*, **49**, 270.
- Cooper, A. C. D., and Johnson, R. H. (1973). The bacteria and parasites found in Batswana children with acute gastroenteritis. *South African Medical Journal*, **47**, 1705.
- Cypess, R. H., Swidwa, D. W., Kenny, J. S., and Yee, R. B. (1974). Influence of Metazoan infection in the mouse on enteric colonization and immune response to *Escherichia coli*. *Journal of Infectious Diseases*, **130**, 534.
- Davidson, G. P., Bishop, R. F., Townley, R. R. W., Holmes, I. H., and Ruck, B. J. (1975). Importance of a new virus in acute sporadic enteritis in children. *Lancet*, **1**, 242.
- Donta, S. T., Sack, D. A., Wallace, R. B., Dupont, H. L., and Sack, R. B. (1974). Tissue-culture assay of antibodies to heat-labile *Escherichia coli* enterotoxins. *New England Journal of Medicine*, **291**, 117.
- Drachman, R. J. (1974). Acute infectious gastroenteritis. *Pediatric Clinics of North America*, **21**, 711.
- Dupont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., LeBrec, E. H., and Kalas, J. P. (1971). Pathogenesis of *Escherichia coli* diarrhoea. *New England Journal of Medicine*, **285**, 1.
- Echeverria, P., Blacklow, N. R., and Smith, D. H. (1975). Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhoea in Boston children. *Lancet*, **2**, 1113.
- Edwards, P. R., and Ewing, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd ed. Burgess, Minneapolis.
- Formal, S. D., Dupont, H. L., and Hornick, R. B. (1973). Enterotoxic diarrheal syndromes. *Annual Review of Medicine*, **24**, 103.
- Freij, L. (1973). Observations on parasitic and bacterial infections of the intestine among paediatric outpatients in Addis Ababa. *Ethiopian Medical Journal*, **11**, 13.
- Gorbach, S. L. (1974). Scatologic aspect of *Escherichia coli*. *Lancet*, **1**, 50.
- Gorbach, S. L., and Khurana, C. N. (1972). Toxinogenic *Escherichia coli*. A cause of infantile diarrhea in Chicago. *New England Journal of Medicine*, **287**, 791.
- Gorbach, S. L., Kean, B. H., Evans, D. G., Evans, D. J., and Bessudo, D. (1975). Travellers' diarrhea and toxinogenic *Escherichia coli*. *New England Journal of Medicine*, **292**, 933.
- Gordon, J. E. (1971). Diarrheal disease of early childhood: world wide scope of the problem. *Annals of the New York Academy of Sciences*, **176**, 9.
- Guerrant, R. L., Moore, R. A., Kirschfeld, P. M., and Sande, M. A. (1975). Role of toxinogenic and invasive bacteria in acute diarrhoea of childhood. *New England Journal of Medicine*, **293**, 567.

- Gyles, C. S. M., So, M., and Falkow, S. (1974). The enterotoxin plasmids of *Escherichia coli*. *Journal of Infectious Diseases*, **130**, 40.
- Heyworth, B., and Brown, J. (1975). Jejunal microflora in malnourished Gambian children. *Archives of Disease in Childhood*, **50**, 27.
- Holmgren, J., Söderlind, O., and Wadström, T. (1973). Cross-reactivity between heat-labile enterotoxins from *Vibrio cholerae* and *Escherichia coli* in neutralization tests in rabbit ileum and skin. *Acta Pathologica et Microbiologica Scandinavica*, **B81**, 757.
- Jelliffe, D. B. (1966). *The Assessment of the Nutritional Status of the Community*. Monograph Series No. 53. World Health Organization, Geneva.
- Kallings, L.-O. (1968). Transport of bacterial specimen. *Biotechnology and Bioengineering*, **10**, 399.
- Klipstein, F. A., Holdeman, L. W., Corcino, J. J., and Moore, W. E. C. (1973). Enterotoxigenic bacteria in tropical sprue. *Annals of Internal Medicine*, **79**, 632.
- Koupal, L. R., and Deibel, R. H. (1975). Assay, characterization and localization of an enterotoxin produced by salmonella. *Infection and Immunity*, **11**, 14.
- Lacey, R. W. (1975). A critical appraisal of the importance of R-factors in the enterobacteriaceae *in vivo*. *Journal of Antimicrobial Chemotherapy*, **1**, 25.
- Mata, L. J., and Urrutia, J. J. (1971). Intestinal colonization of breast-fed children in a rural area of low socioeconomic level. *Annals of the New York Academy of Sciences*, **176**, 93.
- Nalin, D. R., McLaughlin, J. C., Rahaman, M., Yunus, M., and Curlin, G. (1975). Enterotoxigenic *Escherichia coli* and idiopathic diarrhoea in Bangladesh. *Lancet*, **2**, 1116.
- Nord, C.-E., Wadström, T., and Dahlbäck, A. (1975). Evaluation of different diagnostic kits for enterobacteriaceae. *New Approaches to Identification of Micro-organisms*, p. 393. Ed. by L. G. Hedén and T. Illéni. Wiley, New York.
- Pierce, N. F., and Wallace, C. K. (1972). Stimulation of jejunal secretion by a crude *Escherichia coli* enterotoxin. *Gastroenterology*, **63**, 439.
- Ridley, D. S., and Hawgood, B. C. (1956). The value of formalin concentration of faecal cysts and ova. *Journal of Clinical Pathology*, **9**, 74.
- Rudoy, R. C. and Nelson, J. D. (1975). Enteroinvasive and enterotoxinogenic *Escherichia coli*. Occurrence in acute diarrhea of infants and children. *American Journal of Diseases of Children*, **129**, 668.
- Sack, R. B. (1975). Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. *Annual Review of Microbiology*, **29**, 333.
- Sack, R. B., Hirschhorn, N., Brownlee, I., Cash, R. A., Woodward, W. E., and Sack, D. A. (1975). Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. *New England Journal of Medicine*, **292**, 1041.
- Staley, T. E., and Colwell, R. R. (1973). Deoxyribonucleic acid reassociation among members of the genus *Vibrio*. *International Journal of Systemic Bacteriology*, **23**, 316.

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Addendum

Since this paper was completed several studies on the epidemiology of enterotoxinogenic bacteria in infantile diarrhoea in different geographic areas have been published. Guerrant *et al.* (1975) in a hospital in Brazil found 20 instances (50%) of enterotoxinogenic *Esch. coli*, *Klebsiella*, and *Enterobacter* sp. in a group of 40 children with diarrhoea. In a hospital in Texas (Rudoy and Nelson, 1975) enterotoxin-producing *Esch. coli* were found in 36 children (86% of the total) with diarrhoeal disease, but also in 41% of a control group of 17 children, while isolates which produced enterotoxin and were invasive were only found among the children with acute intestinal symptoms. A study of 493 children in Bangladesh with diarrhoea showed that 55% harboured enterotoxinogenic bacteria (Nalin *et al.*, 1975), while in Boston (Echeverria, Blacklow, and Smith, 1975), no enterotoxin-producing bacteria but rotaviruses were reported in 61 of 61 children. These recent observations clearly indicate the need for further work on the epidemiology of acute diarrhoeal disease, and show the impact of enterotoxinogenic bacteria in different geographical areas among different socioeconomic groups (Sack, 1975).