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Escherichia coli O 27 in adult diarrhoea

By BETTY C. HOBBS, B. ROWE, MARGARET KENDALL, P. C. B. TURNBULL and A. C. GHOSH

Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

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SUMMARY

Escherichia coli O 27 H 7 was found in 16 stool samples submitted during a Caribbean cruise (Cruise *Z*) by 29 patients reporting with diarrhoea. A retrospective search revealed *E. coli* O 27 H 7 in 11 of 20 and 2 of 14 stool cultures from patients on two previous cruises (*Y* and *X* respectively) and in a culture from fresh cream (Cruise *Y*). The repeated occurrence of *E. coli* O 27 H 7 in the absence of any other apparent cause suggested that this serotype may have been responsible for the diarrhoea. The results of pathogenicity tests suggested that this strain elaborated heat-stable (ST) enterotoxin.

The possibility that food may have been the vector is discussed.

INTRODUCTION

The belief that *Escherichia coli* caused enteritis only in children under 2 years of age was held for many years; however, recent work has shown that *E. coli* may have an important role in adult diarrhoea. Reports suggest that epidemic infantile enteritis is caused by a restricted range of serogroups (Taylor, 1961) and it seems that a different range of serogroups may be responsible for adult enteritis (Lancet, 1975).

E. coli is a predominant member of the normal intestinal flora in man and animals and therefore its role as an intestinal pathogen has been difficult to prove. The recognition of enteropathogenic *E. coli* in adult diarrhoea has been mostly confined to epidemiologically significant outbreaks where one serotype of *E. coli* was present in pure culture in faecal samples from the majority of patients and in which no other intestinal pathogens were found. In the investigation of such outbreaks a specially prepared diagnostic antiserum to the epidemic serotype may be required. Two such outbreaks were described by Hobbs, Thomas & Taylor (1949) and Rowe, Taylor & Bettelheim (1970).

In the outbreak described by Hobbs, Thomas & Taylor (1949) the faeces were plated on deoxycholate-citrate agar and the predominance of a non-lactose fermenting coliform organism was observed in a number of patients. Subsequently, the coliform was shown to be an atypical *E. coli* belonging to serogroup O 124. Human volunteer experiments showed that the strain caused diarrhoea.

Rowe, Taylor & Bettelheim (1970) studied troops arriving in Aden and

experiencing diarrhoea within a few days after arrival. The *E. coli* in the faeces of healthy soldiers and diarrhoea cases were serotyped and a new and hitherto unidentified serogroup subsequently named O 148 was clearly associated with the diarrhoea. A similar study was made in the Arabian Gulf area (Rowe, 1973) and again *E. coli* O 148 was found to be the epidemic serogroup in the outbreak of diarrhoea. This serogroup was found in food and in the kitchen environment and appeared to be disseminated in the meals. The newly arrived troops experienced the diarrhoea due to *E. coli* O 148 soon after arrival whereas troops who had been in the camp for several months did not have diarrhoea associated with this epidemic serogroup.

An inquiry into the reason for an unusually high and sustained rate of gastro-enteritis in passengers and crew on successive cruises of a large liner led to the investigation reported here.

MATERIALS AND METHODS

Surveillance was made of food and faecal samples taken during ten cruises to Scandinavian, Iberian (including Canary Islands), Mediterranean or Caribbean ports.

All food and faecal samples were obtained on board ship and were examined during the voyage or afterwards in the Food Hygiene Laboratory. Sample containers, media, an improvised incubator and other basic equipment were provided for the work on the ship. When a microbiologist was not available on board, the medical staff inoculated enrichment media, streaked plates and stored them refrigerated for collection at port.

Plating media for stools included horse blood, MacConkey, deoxycholate-citrate, brilliant green, xylose lysine deoxycholate and thiosulphate citrate bile salts sucrose agars. Selenite F in screw-capped bottles was used for enrichment. Incubation temperatures tended to vary somewhat in the improvised incubator used on the ship and stored faeces were frequently re-examined on shore.

When a microbiologist was on the ship, freshly passed stools were examined before treatment was given and information on private medication was recorded. Since there was no provision for carrying out biochemical tests on the ship, suspicious colonies of non-lactose and lactose-fermenting organisms and sweeps of lactose fermenters from apparently pure growth on MacConkey agar plates were transferred to slopes of nutrient agar or Dorset egg and held for subsequent laboratory examination on shore. On arrival at the laboratory all the cultures were streaked on MacConkey agar plates and five individual colonies were subcultured to brilliant green lactose bile broth (BGBB) and peptone water (PW) for incubation at 44 °C. All colonies producing gas in BGBB and indole in PW were considered to be *E. coli* and were examined biochemically using peptone water sugars with Andrade's indicator incubated at 37 °C for 14 days and decarboxylase broths (Møller, 1955) incubated at 37 °C for 4 days. Antibiotic resistance patterns were determined; Diagnostic Sensitivity Test agar plates (Oxoid CM 261) containing

0·25 % horse blood were flooded with 4 hr. nutrient broth cultures. When the plates had dried, Oxoid Multodisk No. 30.44 K and Oxoid single sensitivity disks of nalidixic acid (30 μg.) and furazolidone (100 μg.) were placed on each. After overnight incubation at 37° C, the zones of inhibition were compared with those of strain *E. coli* NCTC 10418. These cultures were also subjected to preliminary serological typing using antisera to six strains whose biochemical patterns were found to be occurring with some frequency.

One hundred and thirty-nine strains were submitted to the Salmonella and Shigella Reference Laboratory for confirmation and complete serological identification. Twenty-nine strains were subjected to pathogenicity tests which included the ligated rabbit ileum ('rabbit loop test' of De & Chatterje, 1953), infant mice (Dean, Ching, Williams & Harden, 1972) and cultured Chinese hamster ovary (CHO) cells (Guerrant *et al.* 1974). One hundred and eighty-five strains were also studied using the biochemical pathogenicity test of Evans & Evans (1973) based on three phenotypic characteristics: utilization of sucrose as sole carbon source, relative growth at pH 8·5 and production of large amounts of ammonium-sulphate-precipitable materials in culture supernatant fluids.

Foods collected from the galley were stored in cold insulated containers until examined, usually within 24 hr. Samples were shaken well with quarter-strength Ringer's solution in a plastic bag and loopfuls were plated out on blood and MacConkey agars; growth was graded from ± to + + + +. Plates of interest were held for full laboratory examination on shore.

Swabs moistened in Ringer's solution were rubbed over surfaces of bowls, churns and cutting boards and cultured on blood agar; the amount of growth was recorded after approximately 24 hr. These plates were usually overgrown on arrival at the laboratory on shore. Using a swab, a heavy inoculum from each plate was transferred to MacConkey broth at 37 °C.; tubes showing acid and gas formation were subcultured to MacConkey agar plates and resulting cultures were processed in the same manner as for faeces.

RESULTS

Comparisons made between the ship in question and other cruise-ships with similar itineraries indicated a high rate of diarrhoea in the former.

Results from bacteriological examination of stool samples were obtained from a number of cruises on this ship. *E. coli* serogroup O 27 was first isolated from patients on the November 1973 Caribbean Cruise (*Z*) on which the complete bacteriological investigation was carried out. Sixty-seven persons, 59 passengers and 8 crew, reported sick with diarrhoea, which was sometimes accompanied by vomiting, abdominal pain or pyrexia. Stool samples were examined from 36 of these persons; 29 with diarrhoea only and the remaining 7 with diarrhoea and vomiting. *E. coli* O 27 was isolated from 16 of the 29 patients with diarrhoea only, but was not isolated from the remaining 7. Actual numbers of strains tested are shown in Table 1.

More epidemiological details of this cruise are given in the paper by Hobbs, Colbourne & Mayner (1975). The duration of illness varied but was usually

Table 1. *Isolation of* Escherichia coli *from stools of passengers reporting with diarrhoea*

		Patients with *E. coli*	No. of *E. coli* strains: Total examined	O 27	Other	No. patients with O 27 and other types	No. types other than O 27
Cruise *X*	Patients with O 27	2	9	9	0	0	0
	Patients with other *E. coli*	8	30	0	30	0	8
	Patients, no *E. coli* found	4	0	0	0	0	0
	Total	14	39	9	30	0	8
Cruise *Y*	Patients with O 27	11	39	29	10	6	7
	Patients with other *E. coli*	7	19	0	19	0	6
	Patients, no *E. coli* found	2	0	0	0	0	0
	Total	20	58	29	29	6	13
Cruise *Z*	Patients with O 27	16	185	162	23	4	5
	Patients with other *E. coli*	17	154	0	154	0	13
	Patients, no *E. coli* found	3	0	0	0	0	0
	Total	36	339	162	177	4	18

2–3 days, and the incidence was highest during the days of sailing through the warm, humid atmosphere of the Caribbean islands. Macroscopically the stools varied from mucoid fluid to soft to well-formed. Parasites were not found in the wet films which were examined. *Salmonella*, *Shigella* and *Vibrio parahaemolyticus* were not found and *Clostridium welchii* levels were normal.

E. coli O 27 was then looked for among the cultures of *E. coli* isolated from similar cases on other cruises and which had been retained in the laboratory. Strains of this serogroup were found in 11 of 18 cases from Cruise *Y* and 2 of 10 from Cruise *X* (Table 1). All *E. coli* O 27 strains from the three cruises produced uniform patterns of biochemical and drug resistance behaviour (Table 2). In addition, identification of the flagellar antigen was carried out on representative O 27 strains from each patient; all were found to be H 7, suggesting that *E. coli* O 27 H 7 was the epidemic aetiological agent.

Food was not definitely established as the agent of dissemination of *E. coli* O 27 H 7; this serotype was isolated from food (fresh cream – Cruise *Y*) once. However, colony and *E. coli* counts in food were often very high, which indicated faults in hygiene and storage. Under these conditions it is possible that strains of *E. coli* O 27 might be missed among the other *E. coli*.

Thirty strains of *E. coli* including 11 strains of *E. coli* O 27 were grown over-

Table 2. *The biochemical and antibiotic susceptibility characteristics of the* Escherichia coli *O* 27 *strain*

Biochemical*		Antibiotic	
Sucrose	AG	Chloramphenicol	S
Sorbose	–	Colistin sulphate	S
Raffinose	AG	Nitrofurantoin	S
Dulcitol	AG	Sulphafurazole	S
Salicin	AG	Kanamycin	S
Arginine dihydrolase	–	Ampicillin	S
Lysine decarboxylase	+	Streptomycin	S
Ornithine decarboxylase	+	Tetracycline	R
		Nalidixic acid 30 μg.†	S
		Furazolidone 100 μg.†	S

AG = Acid and gas produced; S = sensitive; R = resistant.

* Results are given only for tests which are known to be variable with different strains of *E. coli*.

† Single sensitivity disks (Oxoid); remainder on Oxoid Multodisk code 30.44 K.

Table 3. *Tests for enterotoxin production by* Escherichia coli *strains from the three cruises*

	Heat-stable (ST) enterotoxin				Heat-labile (LT) enterotoxin			
	1 kg. rabbit at 7 hr.		Infant mouse		Adult rabbit at 24 hr.		CHO* cell line	
	Tested	Positive	Tested	Positive†	Tested	Positive‡	Tested	Positive
O 27 H 7 Ship strains	11	11	6	6	3	0	3	0
O 27 K?: H- NCTC 9027	1	0	1	0	ND	ND	ND	ND
Other strains	18	1 §	6	0	ND	ND	ND	ND

* Chinese hamster ovary.

† Ratio of intestinal weight to body weight ⩾ 0·08.

‡ Ratio of volume of accumulated fluid to loop length ⩾ 0·3.

§ *E. coli* O 27 was not isolated from this patient, who did not report diarrhoea.

ND = not done.

night at 37° C. in the medium of Evans, Evans & Gorbach (1973), and cell-free filtrates were prepared for enterotoxin tests; the results are summarized in Table 3. All *E. coli* O 27 strains gave positive loops (ratio of volume of accumulated fluid to loop length ⩾ 0·3) after 7 hr. in young rabbits (approximately 1 kg.), six tested in infant mice all gave positive readings (ratio of intestinal weight to body weight ⩾ 0·08). Three of the strains tested in adult rabbits (24 hr. incubation) and a further three tested for their effect on CHO cells all gave negative reactions. Although heat-stability tests were not carried out, these results suggest that these strains of *E. coli* O 27 produced heat-stable (ST) enterotoxin but not heat-labile (LT) enterotoxin (Guerrant *et al.* 1974; Staley, Norris & Smith-Staley, 1974; Sack *et al.* 1975).

With the biochemical pathogenicity test of Evans & Evans (1973), the strains of *E. coli* O 27 H 7 gave a number of different patterns and similarly other *E. coli* from faeces and food gave a variety of patterns. Because of this, it was felt that this test did not appear to be indicative of enteropathogenicity.

Although the association between increase of cases and of temperature and humidity does not suggest virus food poisoning, nine faecal samples were submitted for virus examination. Coxsackie A picorna viruses were found in one sample.

DISCUSSION

There are many published accounts of food-borne illness arising from food eaten on ships, trains, aircraft and in roadside cafés (Roberts & Hobbs, 1974; Hobbs *et al.* 1975; Hobbs, 1976). Various agents of infection have been incriminated, e.g. *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*, although frequently the cause has remained obscure.

In the present investigation the occurrence of *E. coli* O 27 in numerous patients reporting with diarrhoea, for which no other cause was apparent, suggested that strains of this serogroup might have been responsible. Three outbreaks of gastroenteritis due to *E. coli* O 27 are reported in the literature (Sakai *et al.* 1970; Sakazaki, Tamura & Nakamura, 1974; Shore, Dean, Holik & Davis, 1974). This serogroup is rarely found in the U.K. and in this instance may have originated either from the Middle or Far East, perhaps via the galley crew, or from foods taken on board from a number of different countries.

E. coli is one of the commonest intestinal commensal organisms; it is readily spread in the environment and can multiply in a variety of foods. Solodovnikov *et al.* (1974) used *E. coli* M-17 as an indicator organism to demonstrate that food may play an important role in the spread of *E. coli* infection. Although, in the present investigation, *E. coli* O 27 was isolated only once from food, high bacterial and *E. coli* colony counts in many of the foods indicated that hygiene and storage facilities in the galley were inadequate and it is possible that food was the vector. The appearance of *E. coli* on more than one cruise in different regions suggests that infection was acquired on board ship and not at ports of embarkation and call.

Food-borne *E. coli* has previously been implicated in food poisoning outbreaks (Barnard & Callahan, 1971; Schnurrenberger, Beck & Pate, 1971; Marier *et al.* 1973). B. Rowe (personal communication), in a follow-up study of a food-borne outbreak caused by *E. coli* O 148 (Rowe, 1973), found that improved hygiene resulted in a marked reduction in the incidence of diarrhoea. Foods with low bacterial counts (< 100,000/g.) are unlikely to initiate infection (Formal *et al.* 1971); care is necessary to avoid contamination during preparation and to prevent bacterial multiplication during storage. Recommendations on how such difficulties may be avoided are made by Roberts & Hobbs (1974), Hobbs *et al.* (1975) and Hobbs (1976).

E. coli has been used as an indicator organism for faecal pollution and general lack of hygiene in the food industry for many years. The possibility that entero-

pathogenic strains may be present in a particular environment or material should be considered, and surveillance studies may be useful to obtain a better understanding of the origins of enteropathogenic *E. coli* in foods, man and animals and to establish the patterns of spread of such strains.

The chief difficulty in the diagnosis of *E. coli* enteritis in adults is the accurate identification of the organisms and this is essential to establish correlation between patients and foods. Routine laboratories have limited facilities for the examination of strains of *E. coli*. In the search for an epidemic strain from an outbreak the determination of biochemical and drug resistance patterns of strains isolated may be a useful preliminary; the frequent occurrence of strains with common characteristics is suspicious. These studies should be accompanied by serological examination but, though antisera for the infantile enteropathogenic serogroups are readily available, those for the adult serogroups are not. If studies at the routine laboratory suggest an epidemic strain, cultures should be sent to a reference laboratory for accurate serotyping using the complete range of somatic 'O' and flagellar 'H' antigens and should be tested for enterotoxin production and invasiveness.

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