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Studies on the enterotoxigenicity of environmental *Escherichia coli*, belonging to serotypes normally considered enterotoxigenic

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SUMMARY

Fifteen strains of *Escherichia coli* which had been collected in previous studies from animals and meat were studied.

They belonged to serotypes considered enterotoxigenic and were examined for the production of the heat-labile and heat-stable enterotoxins. Only one of these strains (O8.Hnt) isolated from a cowpat in Cheshire produced heat-labile enterotoxin. Another strain (O8.H9) isolated from a cowpat in another part of Cheshire gave results suggesting production of small amounts of the heat-stable enterotoxin. The ecological aspects of these results are discussed.

INTRODUCTION

As there are over 160 internationally accepted 'O' antigens and over 50 internationally accepted 'H' antigens, it is theoretically possible that there might be formed over 8000 'OH' serotypes of *Escherichia coli*. However, in a recent study on the sources of over 20000 strains (Bettelheim, 1978*a*, *b*) only 1173 different 'OH' serotypes were found, and it was noted that certain serotypes appeared to be restricted in habitat.

Ørskov *et al.* (1976) noted that certain 'OH' serotypes predominated among enterotoxigenic *E. coli* isolated from many parts of the world. Of these, O6.H16 and O8.H9 seemed the most common. More recently enterotoxigenic strains of these serotypes have been associated with a number of cases of human diarrhoea (Rowe *et al.* 1978; Ørskov & Ørskov, 1977; Merson *et al.* 1979) including a major waterborne outbreak in U.S.A. (Rosenberg *et al.* 1977).

As a few strains belonging to these and related serogroups had been isolated from environmental sources unrelated to diarrhoeal diseases (Shooter *et al.* 1974; Bettelheim *et al.* 1974, 1976) it was considered that useful ecological information could be obtained if these were assayed for enterotoxigenicity.

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Table 1. *Serotype, source and enterotoxigenicity of strains studied*

E. coli strain	Serotype	Source	Enterotoxin assay: Heat labile	Enterotoxin assay: Heat stable
X194/7/1	06.H16	Chickens	–	–
X212/D/W	06.H16	Chickens	–	–
X209/7/1	06.H16	Chickens	–	–
X212/D/G	06.H–	Chickens	–	–
X208/E/1	R.H16	Chickens	–	–
X208/E/2	R.H16	Chickens	–	–
X208/E/3	R.H16	Chickens	–	–
C72/10	08.H9	Cowpat, Cheshire B*	–	–
C78/3	08.H9	Cowpat, Cheshire A*	–	–
C78/4	08.H9	Cowpat, Cheshire A*	–	weak ±
C72/3	08.Hnt†	Cowpat, Cheshire B*	+	–
C47/1/1	08.H–	Cowpat, Denbighshire	–	–
C47/1/2	08.H–	Cowpat, Denbighshire	–	–
C47/4	08.H–	Cowpat, Denbighshire	–	–
C47/9	08.H–	Cowpat, Denbighshire	–	–

* Two different sites in Cheshire. † Untypable with 55 H-antisera.

MATERIALS AND METHODS

Bacterial strains

The test strain for both enterotoxin assays was *E. coli* H-10407 (O78.H11). It had been obtained from Dr D. J. Evans Jr. (University of Texas Medical School at Houston, Houston, Texas U.S.A.). The test strains, their serological structure and source are listed in Table 1.

Heat-labile enterotoxin assay

The test strains were cultured from stock nutrient agar slopes into 5 ml syncase broth (Sack & Sack, 1975) in 1·5 cm diameter test-tubes with loose fitting steel caps. The cultures were incubated at 37 °C on a tube roller (New Brunswick Scientific Co., Model TC-1) as recommended by I. K. Wacksmuth (personal communication). They were subcultured into fresh syncase broth, similarly incubated, and a supernatant was prepared and assayed by methods based on the work of Sack & Sack (1975) and current practice at the Center for Disease Control, Atlanta, GA, U.S.A. The Y – 1 adrenal cell-line (CCL-79) was obtained from the American Type Culture Collection.

Heat-stable enterotoxin assay

The culture filtrates obtained for the heat-labile enterotoxin assay were also used for the heat-stable assay. The method of Dean *et al.* (1972) was used, employing 3 to 4-day-old mice belonging to the New Zealand National Health Institute strain.

RESULTS

The results, which are given in Table 1, show that only one strain (C72/3) produced the heat-labile enterotoxin, and another strain (C78/4) gave a variable weakly-positive response in the heat-stable enterotoxin assay.

DISCUSSION

Ørskov *et al.* (1976), in their studies on special *E. coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children, suggested that some of the more prevalent serotypes (06.H16, 08.H9, O78.H11) 'represent clones which have been selected to the special conditions in the small intestine and selected to carry the plasmids necessary to provoke diarrhoea.' Although rare in the environment, these serotypes have nevertheless been found in sites unrelated to human diarrhoea (Bettelheim, 1978*a*, *b*).

The present study shows that only one of these strains produces the heat-labile enterotoxin, and another gave a weak reaction for the heat-stable enterotoxin. However, it does suggest that these special serotypes, occasionally harbouring plasmids for enterotoxigenicity, occur in the environment where they represent a potential source of infection. These strains had been isolated many years ago and might have lost their enterotoxigenicity in culture. Nevertheless, that two out of 15 were still enterotoxigenic is considered significant for environmental strains.

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