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A study of enterotoxigenic *Escherichia coli*, serogroup 0126, by bacterial restriction endonuclease DNA analysis (BRENDA)

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SUMMARY

Sixteen isolates of *Escherichia coli* were subjected to bacterial restriction endonuclease DNA analysis (BRENDA). Nine of these isolates were from an outbreak of human diarrhoea and produced stable toxin, the remaining seven were non-toxigenic strains from animal and human sources. The isolates from the outbreak produced indistinguishable DNA electrophoretic patterns in spite of their assignment to seven different H serotypes. Their BRENDA patterns were markedly different from the other isolates examined. These results support the epidemiological evidence that a single-strain outbreak had occurred, and they cast doubt on the value of H typing for this particular investigation.

INTRODUCTION

Bettelheim & Reeve (1982) reported an outbreak of gastroenteritis, which affected both adults and children, from which they isolated *Escherichia coli* belonging to serogroup 0126. All the outbreak-strains produced the heat-stable enterotoxin ST. In spite of having been isolated from a group of patients that could be epidemiologically linked, these organisms displayed a wide range of H antigen types. No definitive conclusion was drawn from the results of the H typing, but it was proposed that either more than one toxigenic strain was responsible or that only one epidemic strain was involved and that the H antigen serotyping was, in this instance, misleading.

More recently a method of subspecies differentiation of bacteria has been developed using restriction endonuclease analysis of bacterial DNA. Total DNA patterns from individual isolates are viewed and compared following digestion with restriction endonucleases and electrophoresis in agarose gels (Marshall, Wilton & Robinson, 1981; Robinson *et al.* 1982). This method, which has been given the acronym BRENDA, has already shown close agreement with the established

serological typing scheme used for *Leptospira interrogans*. The aim of the present study was to use the BRENDA technique to try to resolve the apparent conflict in this outbreak between the H antigen serotyping results and the available epidemiological evidence.

MATERIALS AND METHODS

Bacterial isolates

A total of 16 type 0126 *E. coli* were examined. Nine of these isolates were enterotoxigenic and were derived from patients involved in an outbreak of gastroenteritis (Bettelheim & Reeve, 1982); four were non-toxigenic isolates obtained from calves at a slaughterhouse where a number of the patients worked. Three isolates from babies were unrelated to the outbreak of gastroenteritis and had been submitted for routine typing.

Serotyping

All isolates were serotyped with *E. coli* O antisera (O 1–O 166) and H antisera (H 1–H 56) as previously described (Bettelheim & Reeve, 1982).

Enterotoxin testing

The isolates were tested for the production of heat-labile toxin (LT) using the Y-1 adrenal cell ássay based on a modified version (Bettelheim *et al.* 1980) of that devised by Sack & Sack (1975), and heat-stable toxin (ST) with the suckling mouse assay (Giannella, 1976). The nine isolates recovered during the outbreak were tested at the time of the original investigation and again after this laboratory investigation had been carried out.

Preparation of DNA

The DNA was extracted by the method of Marshall, Wilton & Robinson (1981). After extraction each sample was dialysed exhaustively against TE (10 mM Tris-HCl, pH 7·5, and 1 mM EDTA) at 4 °C. The absorbance at 260 nm (Unicam spectrophotometer, SP 500) of each preparation was measured in a quartz-glass cell with a 1 cm light path to obtain the nucleic acid concentration; it was found that one optical density unit was equivalent to 50 μg/ml of double-stranded DNA. To calculate the percentage of RNA, the DNA concentration of each sample was also measured by fluorimetry (Le Pecq & Paoletti, 1966) using calf thymus DNA (type 1, Sigma Chemical Co., St Louis, Missouri, USA) to construct a standard curve 0–1 μg/ml of DNA.

Restriction endonuclease digestion of DNA

Two μg of bacterial DNA was completely digested at 37 °C for 1 h, with two to five units of restriction endonuclease $EcoR_1$, (New England Biolabs, Beverly, MA 01915, USA) using enzyme buffer made according to the manufacturer's specifications.

Gel electrophoresis and photography

Gel electrophoresis and photography were performed according to the method of Marshall *et al.* (1981) with the exception that electrophoresis was maintained

Table 1. *The isolates of* E. coli *used in this study grouped according to their BRENDA patterns*

Source	Date of isolation	Serotype	Enterotoxin
Patient*			
A	5 Mar. 1981	O126 H9	ST
B	6 Apr. 1981	O126 H19	ST
C	29 Mar. 1981	O126 —	ST
D	13 Apr. 1981	O126 H7	ST
E	16 Apr. 1981	O126 H9	ST
F	24 Apr. 1981	O126 Hr	ST
G	23 Apr. 1981	O126 Hnt	ST
H	24 Apr. 1981	O126 H54	ST
L	2 June 1981	O126 Hr	ST
From meat of Bobby calf 2	28 Aug. 1981	O126 H22	NT
From meat of Bobby calf 3	9 Sept. 1981	O126 H33	NT
From meat of Bobby calf 4	9 Sept. 1981	O126 H33	NT
From faeces of Bobby calf 5	9 Sept. 1981	O126 H33	NT
Baby 1	29 July 1980	O126 Hnt	NT
Baby 2†	20 Feb. 1981	O126 H29	NT
Baby 3	29 June 1981	O126 H29	NT

* All patients had the same BRENDA pattern.
† Both babies had the same BRENDA pattern.

at 5 V/cm until the tracking dye, bromophenol blue, had travelled a distance of 12 cm; this took approximately 3·5 h.

RESULTS

The nine *O126 E. coli* isolates from the outbreak patients (A–H and L) gave indistinguishable BRENDA patterns when digested with the restriction endonuclease $EcoR_1$ (Table 1 and Fig. 1). An outstanding feature of these nine isolates, when compared with the other isolates examined, was their apparent lack of small plasmids as demonstrated when gels were run using undigested DNA. However, a single large-molecular-weight plasmid was revealed when bacterial lysis and electrophoresis were performed according to the Eckhardt method (Eckhardt, 1978).

The four serotype O126 non-toxigenic isolates from meat or animal faeces all gave patterns totally different from the isolates involved in the outbreak. The animal isolates produced patterns which although very similar were not identical to each other (not shown) and each isolate carried a different complement of plasmids as demonstrated by the electrophoresis of undigested DNA. It was not determined if the difference between the patterns of these isolates resulted solely from the different plasmids carried.

Two of the three isolates from babies gave BRENDA patterns indistinguishable from each other (Fig. 1, Lanes 3 and 4), the third baby strain produced a unique pattern (Fig. 1, Lane 6).

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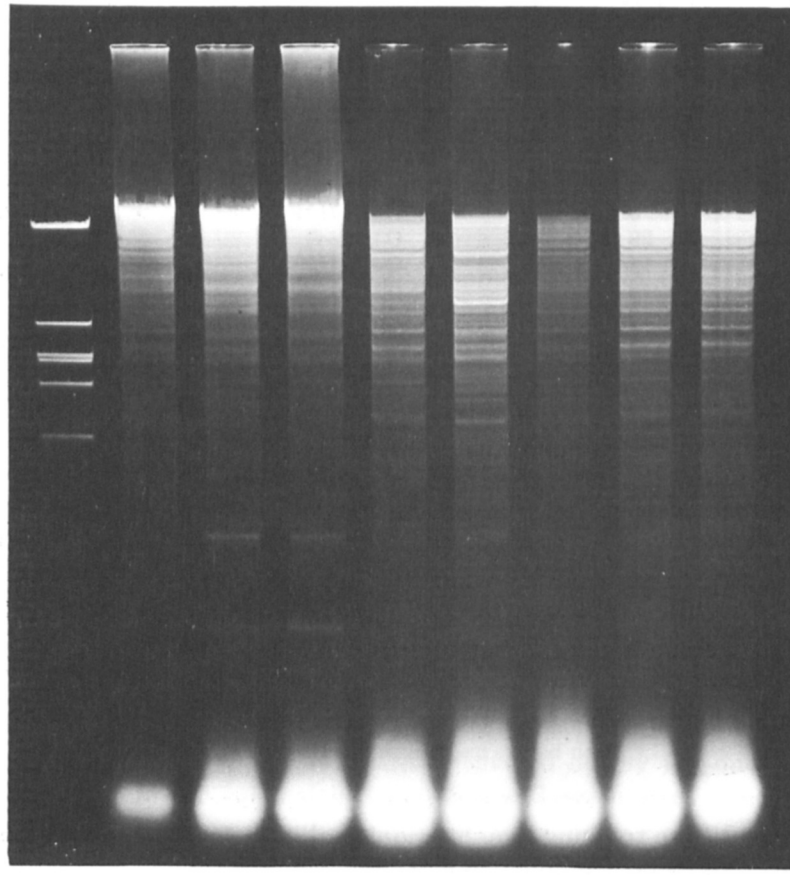

Fig. 1. Gel electrophoresis of cleaved DNA. Lane 1, lambda DNA. Lanes 2, 5, 7, 8 and 9 show the DNA patterns of O 126 *E. coli* from patients involved in the outbreak. Lanes 3, 4 and 6 are O 126 *E. coli* isolated from babies not associated with the outbreak. Lanes 2, 3 and 4 have some unresolved DNA at the top of each pattern.

DISCUSSION

The most widely used markers for epidemiological investigations are usually those stable characters which are under chromosomal control. Serotyping, utilizing both the somatic and flagella antigenic properties of different strains of *E. coli*, has been widely used as a basis for epidemiological studies (Berry, Bettelheim & Gracey, 1983; Bettelheim & Reeve, 1982; Kelly & Smith, 1970; Merson *et al.* 1980). More recently, efforts have been made to correlate the production of heat-labile and heat-stable enterotoxins with specific serogroups and serotypes (Bettelheim *et al.* 1980; Goldschmid & Dupont, 1976; Scotland *et al.* 1981). Extra-chromosomal markers are also being used to differentiate strains of *E. coli*, and markers such as colicin production (Fredericq, Betz-Bareau & Nocolle, 1956) and R plasmids have been used for epidemiological studies. A new approach to the study of plasmids has been made possible by the discovery of endonucleases which recognize specific cleavage sites (Kelly & Smith, 1970; Nathans & Smith, 1975). The first to use this approach in an epidemiological investigation were Sadowski *et al.* (1979),

who demonstrated that some plasmids are sufficiently stable to be used as epidemiological markers for at least a few months after an outbreak. The usefulness of plasmids for strain differentiation was also demonstrated in this present study, where plasmid profiles enabled the animal isolates of O126 and H33 to be differentiated one from another.

The method of subspecies differentiation using restriction endonucleases to digest the total bacterial DNA is not reliant on the presence of plasmids and produces a fingerprint pattern composed of both chromosomal and plasmid DNA characteristic for that strain. The technique has been used for the identification of leptospiral serovars by Marshall, Wilton & Robinson (1981) and Robinson *et al.* (1982). Kaper *et al.* (1982), Bradbury *et al.* (1984) and Kakoyiannis *et al.* (1984) used the technique for epidemiological studies of *Vibrio cholerae*, *Campylobacter jejuni* and *C. coli*, respectively.

A surprising feature of our work on this epidemic strain was our initial failure to demonstrate any plasmid, since the production of heat-stable enterotoxin in *E. coli* is normally under plasmid control. However, in further work not detailed here using the Eckhardt method it was possible to show that this strain did have a single large-molecular-weight plasmid. Plasmid control of ST production in these organisms cannot therefore be discounted.

The indistinguishable nature of the BRENDA patterns produced by the nine outbreak isolates of *E. coli* O126, and their dissimilarity to patterns produced by both human and animal non-enterotoxigenic O126 strains, provides strong evidence that they were indeed different isolations of one epidemic strain. These results support the epidemiological evidence but cast doubt on the value of H typing in this particular outbreak. Although the results of this study are from an investigation of limited size, the usefulness of H typing for establishing the identity of field strains of *E. coli* is called into question.

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