Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2

By S. M. SCOTLAND, G. A. WILLSHAW, H. R. SMITH AND B. ROWE

Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, UK

(Accepted 22 June 1987)

SUMMARY

Fifty-four strains of *Escherichia coli* belonging to serogroup O157 were examined for the production of Vero cytotoxins VT1 and VT2, and for other properties such as plasmid content, resistance to antimicrobial agents and colicin production. Twenty-six strains from cases of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in humans produced VT. By serum neutralization tests and hybridization with DNA probes for VT1 or VT2, three classes were recognized which produced either VT1 alone or VT2 alone or both VT1 and VT2. These strains were of H type 7 or non-motile. The strains producing VT were sensitive to all the antimicrobial agents tested, and all carried at least one plasmid which had a molecular weight of $c. 60 \times 10^6$. Seven strains of porcine origin and 21 strains of human origin did not produce VT or hybridize with either DNA probe. None of these strains was of H type 7. Of the 21 human VT⁻ strains, 17 were of extra-intestinal origin and 18 were of H type 45. Twenty-three of the 28 VT⁻ strains were resistant to at least one antimicrobial agent.

INTRODUCTION

Escherichia coli of serogroup O157 was first isolated from piglets with enteritis (Furowicz & Ørskov, 1972). Strains of this serogroup are now recognized as a cause of enteric colibacillosis in piglets; these pathogenic strains may possess the K88 adhesin and produce heat-labile enterotoxin, LT, and heat-stable enterotoxins ST_A and ST_B (Wilson & Francis, 1986 and review by Morris & Sojka, 1985). In humans, strains of this serogroup cause haemorrhagic colitis (HC) (Johnson, Lior & Bezanson, 1983; Riley *et al.* 1983; Pai *et al.* 1984; Remis *et al.* 1984; Ratnam & March, 1986; Smith *et al.* 1987) and haemolytic uraemic syndrome (HUS) (Karmali *et al.* 1985; Neill, Agosti & Rosen, 1985; Gransden *et al.* 1986; Spika *et al.* 1986; Scotland *et al.* 1987). These strains are of flagellar type H7 or non-motile, do not possess the K88 adhesin and do not produce LT or ST. However, they produce a cytotoxin detected on Vero cells termed Vero cytotoxin or VT (Konowalchuk, Speirs & Stavric, 1977).

Two distinct Vero cytotoxins were produced by strains of *E. coli* O157 from cases of HC and HUS (Scotland, Smith & Rowe, 1985), and these were termed

VT1 and VT2. Serum neutralization experiments showed that VT1 was immunologically related to Shiga toxin; O'Brien & LaVeck (1983) had previously demonstrated this for the VT produced by strains of *E. coli* O26. VT2 was not immunologically related to Shiga toxin, and some strains of *E. coli* O157 produced only VT2. Other O157 strains produced both VT1 and VT2. The terms Shiga-like toxin I (SLTI) and SLTII have also been used to describe the two toxins (Strockbine *et al.* 1986). VT is phage-encoded in several strains of *E. coli* O157 (Smith *et al.* 1984; O'Brien *et al.* 1984) and DNA sequences coding for VT have been cloned from these phages (Newland *et al.* 1985; Willshaw *et al.* 1985). DNA probes specific for VT1 and for VT2 were developed from these sequences, and these probes show no cross-hybridization under stringent conditions (Willshaw *et al.* 1987).

In this paper *E. coli* belonging to serogroup O157 and isolated from various sources have been compared. They include strains of animal origin and strains of human origin from HC and other diarrhoeal disease, cases of HUS and extra-intestinal infections. The strains were tested for production of VT and for hybridization with DNA probes for VT1 or VT2. Other properties were compared, including resistance to antimicrobial agents and plasmid profiles.

METHODS

Bacterial strains

Strains from the culture collection of the Division of Enteric Pathogens belonging to *E. coli* serogroup O157 were studied. They had been serotyped using antisera for *E. coli* somatic (O) antigens 1-170 and flagellar (H) antigens 1-56 (Gross & Rowe, 1985). These strains had been stored on Dorset egg agar medium at room temperature.

Twenty-one strains of *E. coli* O157 producing VT (VT⁺) had been isolated in the United Kingdom (Table 1). They were compared to four strains isolated in Canada sent by H. Lior and strain 933 sent by A. D. O'Brien (Table 1). Strain 933 was the causative agent of an outbreak of HC in the United States (O'Brien *et al.* 1983).

Strains belonging to serogroup O157 that were isolated in the United Kingdom from human sources but did not produce VT (VT⁻) were also examined (Table 2). These 21 strains had been sent to this laboratory for routine serotyping between 1977 and 1986. Seventeen of these 21 VT⁻ strains were of extra-intestinal origin.

Seven strains belonging to serogroup O157 and isolated from animals were also examined (Table 3). One of these was strain A2, *E. coli* O157.K88ac.H19, the antigenic test strain for serogroup O157 (Furowicz & Ørskov, 1972), which had been isolated from a piglet with enteritis (Sweeney, 1970). A further six O157.K88ac porcine strains were received from C. Wray.

A strain of *Shigella dysenteriae* type 1, E7926, and two derivatives of *E. coli* K12, 60R344 and 60R366, were used as control strains in neutralization tests of VT. Strain 60R366 produced VT1 after acquisition of a recombinant plasmid containing genes cloned from a VT1-encoding phage from strain E30480, *E. coli* O157.H7 (Scotland, Smith & Rowe, 1985; Willshaw *et al.* 1985, 1987). Strain

60R344 produced VT2 after infection with a VT-encoding phage from strain E32511, *E. coli* O 157. H⁻ (Smith *et al.* 1984; Scotland, Smith & Rowe, 1985).

Biochemical tests

The strains were tested for their ability to utilize mucate and to ferment adonitol, arabinose, cellobiose, dulcitol, glucose, glycerol, inositol, inulin, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sorbose, sucrose, trehalose and xylose. They were also tested on MacConkey-sorbitol agar, in which sorbitol (1%) replaced lactose.

Colicin tests

Strains were tested for production of colicins by the overlay test using the colicin-producing or colicin-insensitive strains described by Pugsley (1985), which were received from V. Hughes (Plasmid Section, National Collection of Type Cultures).

Tests for resistance to antimicrobial agents

Strains were tested by the method of Anderson & Threlfall (1974) for resistance to amikacin, ampicillin, chloramphenicol, colomycin, gentamicin, kanamycin, mecillinam, nalidixic acid, netilmicin, spectinomycin, streptomycin, sulphathiazole, tetracyclines, tobramycin and trimethoprim.

Tests for toxin production and invasion of cells grown in tissue culture

The strains were grown as shaken cultures in trypticase soy broth with dextrose (BBL), and sterile culture supernatants were tested for VT as described previously (Scotland, Day & Rowe, 1980). Production of heat-labile enterotoxin (LT) was determined by testing the same sterile culture supernatants in a Y1 adrenal cell test, or by an enzyme-linked immunoassay (ELISA) (Scotland, Gross & Rowe, 1985). The sterile culture supernatants were tested also for heat-stable enterotoxin (ST_A), using the infant mouse test (Dean *et al.* 1972). Because piglets are needed to test for ST_B in intestinal loops we did not test for this toxin. Strains were tested for their ability to invade HEp-2 cells by the method of Scotland, Gross & Rowe (1985).

Neutralization of Vero cytotoxin

Neutralization tests were performed on filtered culture supernatants as described previously (Scotland *et al.* 1987). Polyclonal antisera raised in rabbits against Shiga toxin (VT1) and against VT2 were used separately and together for their ability to neutralize VT. The antiserum to purified Shiga toxin was kindly provided by A. D. O'Brien. The antiserum to VT2 was prepared using VT2 produced by strain E32511 (Scotland *et al.* 1987).

Characterization of plasmids

The plasmid content was determined by agarose gel electrophoresis (Willshaw, Smith & Anderson, 1979) of DNA prepared by the method of Birnboim & Doly (1979). The agarose concentration was 0.6% and molecular weights were measured relative to standard plasmids run on the same gel.

DNA hybridization experiments

Broth cultures were spotted on nylon disks (Hybond-N, Amersham) supported on MacConkey agar plates, which were incubated at 37 °C for 5–6 h. Filters were prepared for colony hybridization as described by Maniatis, Fritsch & Sambrook (1982). The conditions for hybridization and washing at high stringency were as described by Willshaw *et al.* (1985). The probe for VT1 sequences was a 0.75 kb *HincII* fragment contained in a recombinant plasmid (NTP705) that was derived from the VT1 phage in strain H19, serotype O26.H11 (Willshaw *et al.* 1985). The VT2 probe was a 0.85 kb *AvaI-PstI* fragment obtained from a recombinant plasmid (NTP707) containing cloned VT2 genes from a phage originating in strain E32511 (Willshaw *et al.* 1987).

RESULTS

Production of Vero cytotoxins, VT1 and VT2, and hybridization with specific DNA probes

Twenty-one strains of *E. coli* O157 isolated from cases of HUS, HC or diarrhoea in the UK were shown to produce VT by testing culture supernatants (Table 1). All strains were of H type 7 with the exception of two non-motile strains. All hybridized with at least one of the two DNA probes specific for VT genes, and the strains fell into three classes. A single strain of the first class hybridized with only the VT1 probe, 11 strains of the second class hybridized with only the VT2 probe and 9 strains of the third class hybridized with both probes. The 5 VT⁺ strains from Canada and the United States hybridized with both probes. The 21 VT⁺ strains isolated in the UK did not produce ST_A or LT, and this agrees with reports on strains isolated in North America (Johnson, Lior & Bezanson, 1983; Wells *et al.* 1983).

Twenty-one strains of serogroup O157 isolated from human sources did not produce VT or hybridize with either of the VT probes. These strains did not produce ST_A or LT. Eighteen of these strains were of flagellar type H45 (Table 2).

The seven porcine strains of serogroup O157 (Table 3) did not produce VT or hybridize with either of the VT probes. All produced LT detected in both an ELISA and the tissue culture test, but they did not produce ST_A.

Serum neutralization tests

Neutralization tests were performed on VT present in filtered culture supernatants. Table 4 shows the results of neutralization tests with 14 strains of serogroup O157 and the control strains.

VT produced by control strains E7926 and 60R366 and by strain E40705, which hybridized only with the VT1 probe, was neutralized by the anti-Shiga toxin but not by the antiserum raised against VT2. VT produced by strain 60R344 and the five O157 strains that hybridized with only the VT2 probe was neutralized by the anti-VT2 serum but not by anti-Shiga toxin.

Anti-Shiga toxin, when tested alone, did not neutralize the VT produced by any of the eight strains hybridizing with probes for VT1 and VT2. There was a

Table 1. *Properties of strains of E. coli* O157 producing *Vero cytotoxin* (VT)

Strain no.	Year and place of isolation	Clinical details*	H type†	Colicin production‡	Hybridization with probe specific for§		Molecular weight of plasmids carried ($\times 10^6$)	Reference
					VT1	VT2		
E40705	1986 Hereford	HC	H7	D	+	—	59 4.8	Smith <i>et al.</i> 1987
E27164	1981 Bradford	Diarrhoea	H7	D	—	+	61 5.4	Day <i>et al.</i> 1983
E29962	1983 Wolverhampton	HUS outbreak	H7	—	—	+	59	Taylor <i>et al.</i> 1986
E30138	1983 Wolverhampton	HUS outbreak	H7	I	—	+	56	Taylor <i>et al.</i> 1986
E30979	1983 Peterborough	HUS	H7	—	—	+	59 38	Scotland <i>et al.</i> 1987
E32511	1983 Birmingham	HUS	H—	—	—	+	55 36	Scotland <i>et al.</i> 1987
E35413	1984 Ipswich	HUS	H7	—	—	+	60 40	Scotland <i>et al.</i> 1987
E36303	1984 Burton	HUS	H7	—	—	+	57 39	Scotland <i>et al.</i> 1987
E36316	1984 London	HUS	H7	—	—	+	57 2.5	Scotland <i>et al.</i> 1987
E36320	1984 London	HUS	H7	—	—	+	57	Scotland <i>et al.</i> 1987
E36419	1984 Yarmouth	HUS	H7	—	—	+	58	Scotland <i>et al.</i> 1987
E37709	1984 Birmingham	HUS	H7	—	—	+	56 37	Scotland <i>et al.</i> 1987
E22826	1982 High Wycombe	Diarrhoea	H—	—	+	+	59	Day <i>et al.</i> 1983
E30228	1983 Salisbury	HUS	H7	D	+	+	59 40	Scotland <i>et al.</i> 1987
E30480	1983 Wolverhampton	HC	H7	—	+	+	61 5.0	Scotland <i>et al.</i> 1985
E34500	1984 London	HUS	H7	D	+	+	55 4.7	Scotland <i>et al.</i> 1987
E36307	1984 Cardiff	HUS	H7	D	+	+	60 4.7	Scotland <i>et al.</i> 1987
E37719	1984 Birmingham	HUS	H7	—	+	+	61 24	Scotland <i>et al.</i> 1987
E38848	1985 Birmingham	HUS	H7	D	+	+	57 4.5	Scotland <i>et al.</i> 1987
E39047	1985 Norwich	HC outbreak	H7	D	+	+	63 4.8	Palmer, 1986
E39146	1985 Cambridge	HC outbreak	H7	D	+	+	57 4.5	Palmer, 1986
80-2740	1982 Canada	HC	H7	D	+	+	59 4.7	Johnson <i>et al.</i> 1983
81-110	1982 Canada	HC	H7	D	+	+	59 4.7	Johnson <i>et al.</i> 1983
83-1933	1982 Canada	HC outbreak	H7	D	+	+	61 4.7	Johnson <i>et al.</i> 1983
82-1947	1982 Canada	HC outbreak	H7	D	+	+	61 4.7	Johnson <i>et al.</i> 1983
933	1982 United States	HC outbreak	H7	—	+	+	57 2.0	O'Brien <i>et al.</i> 1983

* HC, haemorrhagic colitis; HUS, haemolytic uraemic syndrome.

† H—, indicates non-motile strain.

‡ Letters indicate identified colicin; + indicates colicin which could not be identified; — indicates no colicin produced.

§ + indicates hybridization; — indicates that hybridization was not detected.

Table 2. *Properties of human strains of E. coli 0157 that do not produce VT*

Strain no.	H type*	Source of specimen	Age of patient	Colicin* production	Drug resistance†	Molecular weight of plasmids carried ($\times 10^6$)‡
E10411	H45	Blood	52	—	Ap	94, 60, 28, 26, 9, 5.1, 3.9
E11547	H45	Blood	74	—	Ap	94, 27, 24, 4.4, 3.3
E12734	H45	Blood	44	—	Ap, Nx	86, 5.5, 3.6
E14080	H45	Blood	6	I	Cm, Tc	86, 63
E15746	H45	Blood	Unknown	—	None	94, 3.3, 3.1
E20597	H45	Blood	72	I	Ap	83, 60, 6.8, 3.4
E22493	H45	Blood	59	+	Ap	120, 91, 63, 56, 28, 25, 7.2, 3.7, 2.0
E27236	H45	Blood	48	—	Ap, Sm, Su	95, 60, 30, 3.7, 3.4
E28688	H45	Blood	68	I	None	150, 97, 63, 6.5, 3.7
E11544	H45	Eye	9 days	—	Ap, Sm, Su	94, 58, 3.8
E12506	H45	Eye	4 days	—	None	86, 4.2, 3.2
E9818	H45	Faeces	5 months	—	Ap, Su	100, 55, 25, 4.2, 3.9
E17097	H45	Faeces	3 months	+	Ap, Sm, Su	85, 54, 28, 23, 5.6, 3.3
E24005/2	H—	Faeces	3 months	—	Ap, Cm	3.4
E30288	H45	Faeces	19	—	Ap	100, 53, 7.4, 3.5
E10376	H45	Urine	'Adult'	—	Ap	94, 3.9, 3.2
E13639	H45	Urine	Unknown	—	Ap	86, 52, 5.5, 3.6
E16159	H45	Urine	77	—	None	83, 3.6
E18044	H45	Urine	11 months	+	Ap	71, 60, 7.4, 4.5
E19221	H—	Urine	'Child'	—	Ap	81, 5.9, 3.6
E27023	H39	Urine	2 weeks	—	Ap, Tm	None

* See footnotes to Table 1.

† Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nx, nalidixic acid; Sp, spectinomycin; Sm, streptomycin; Su, sulphathiazole; Tc, tetracyclines; Tm, trimethoprim.

‡ Molecular weights correspond to plasmid DNA bands visible on agarose gels. Bands of molecular weight $< 10 \times 10^6$ may not represent independent plasmids.

Table 3. *Properties of porcine strains of E. coli O157 producing LT but not VT*

Strain no.	H type*	Source of specimen	Colicin production*	Drug resistance†	Molecular weight of plasmids carried ($\times 10^6$)†
A2	H19	Faeces	—	None	110, 90, 52
E39226	H—	Faeces	+	Ap, Km, Sm, Su, Sp, Tc	70, 61, 45, 3·2, 2·7
E39227	H43	Faeces	—	Sm, Su, Sp, Tc	58, 52, 45, 3·0
E39230	H43	Faeces	—	Km, Sm, Su, Sp, Tc	63, 58, 22, 3·1
E39231	H43	Faeces	—	Km, Sm, Su, Sp, Tc	63, 56, 43, 3·1, 1·9
E39232	H43	Faeces	+	Sm, Su, Sp, Tc	58, 52, 45, 2·9
E39233	H43	Faeces	—	Cm	72, 58, 52, 40

* See footnotes to Table 1. † See footnote to Table 2.

Table 4. *Neutralization of Vero cytotoxin*

Strain no.	Hybridization with probe for		Titre of Vero cytotoxin activity*			
	VT 1	VT 2	Without antiserum	With antiserum to		With both antisera
				Shiga toxin (VT1)	to VT2	
<i>E. coli</i> O 157						
E40705	+	—	1250	50	1250	50
E29962	—	+	6250	6250	0	n.t.†
E30138	—	+	6250	6250	0	n.t.
E30979	—	+	6250	6250	0	n.t.
E32511	—	+	31250	31250	50	50
E36419	—	+	6250	6250	0	n.t.
82-1933	+	+	156250	156250	1250	50
E30228	+	+	781250	781250	1250	250
E30480	+	+	781250	781250	6250	250
E36307	+	+	781250	781250	6250	50
E37719	+	+	31250	31250	31250	0
E39047	+	+	31250	31250	31250	0
E39146	+	+	31250	31250	31250	0
933	+	+	31250	31250	1250	50
<i>K12</i> derivatives						
60R344	—	+	781250	781250	0	n.t.
60R366	+	—	156250	6250	156250	6250
<i>Sh. dysenteriae</i> type 1						
E7926	+	—	31250	250	31250	250

reduction in the titre of VT produced by five of these eight strains in the presence of the antiserum against VT 2. For all eight strains the lowest titre was seen when anti-Shiga toxin and antiserum to VT 2 were used together. It was concluded that these eight strains produced both VT 1 and VT 2 but that in five supernatants there was more VT 2 than VT 1. Two additional culture supernatants were prepared at different times with four strains (E30228, E30480, 82-1933 and 933), hybridizing with both VT 1 and VT 2 probes to see whether the proportion of VT 1 to VT 2 was the same in different preparations of the same strain. Anti-Shiga toxin alone did not neutralize any of the eight preparations. The preparations from strains E30228, E30480 and 933 gave the same results as shown in Table 4, that is, partial neutralization with the anti-VT 2 serum and a further drop in titre when both antisera were tested together. One of the additional preparations of strain 82-1933 gave the same results as shown in Table 4; however, the second preparation was only neutralized when both antisera were used together. Thus the proportions of VT 1 and VT 2 produced by a strain varied even when the same procedure was used.

Biochemical tests

All the *E. coli* O 157 strains utilized mucate and fermented arabinose, glucose, glycerol, lactose, mannitol, maltose, sorbose, rhamnose, trehalose and xylose. None fermented adonitol, cellobiose, inositol, insulin or salicin. The strains differed in the fermentation of dulcitol, raffinose, sorbitol and sucrose to give three biogroups.

The 26 VT⁺ strains did not ferment sorbitol within 1 day, although only six strains failed to ferment sorbitol within 14 days. Nevertheless, all the 28 VT⁻ strains fermented sorbitol within 1 day and so the VT⁺ and VT⁻ strains were clearly distinguishable on MacConkey-sorbitol agar plates when these were examined after overnight incubation. This is in agreement with the results of other groups, who have used modifications of this test to screen for VT⁺ O 157 strains (Farmer & Davis, 1985; March & Ratnam, 1986).

Plasmid profiles

All the VT⁺ strains isolated in the UK carried a plasmid with a molecular weight between 55×10^6 and 63×10^6 (Table 1). A similar plasmid had been reported in the VT⁺ strains from Canada (Johnson, Lior & Bezanson, 1983) and the United States (Wells *et al.* 1983) and this was confirmed (Table 1). For some strains this was the only plasmid, but the single VT1⁺ strain and 12 of 14 VT1⁺/VT2⁺ strains also carried a plasmid with a molecular weight of *c.* 4.7×10^6 . All but four of the VT⁻ strains carried at least three plasmids, and the strains gave few common patterns (Tables 2 and 3).

Resistance to antimicrobial agents and other properties

The VT⁺ strains were sensitive to all the antimicrobial agents tested. In contrast, 17 of the 21 VT⁻ strains from human sources and 6 of the 7 LT⁺ porcine strains were resistant to one or more antimicrobial agent. The resistance patterns are shown in Tables 2 and 3.

Thirteen of the 26 VT⁺ strains produced a colicin, and for all except strain E30138 this could be identified as colicin D (Table 1). Strain E30138 probably

Table 5. *Fermentation patterns of strains of E. coli* O 157

No. of strains	Production of		Fermentation of			
	VT	LT	Sorbitol	Dulcitol	Raffinose	Sucrose
26	+	—	(d)	+	+	+
19	—	—	+	(d)	—	—
2*	—	—	+	+	+	+
7	—	+	+	+	+	+

—, No fermentation (14-day test); +, fermentation within one day; (d), variable reaction but all were negative at 1 day.

* These two strains were E27023, H type 39 and E24005/2, which was non-motile.

produced more than one colicin. Eight of the 28 VT[—] strains produced colicins (Tables 2 and 3), but where these could be identified they were not colicin D.

None of the VT⁺ or VT[—] O 157 strains in the present study was invasive in the HEp-2 cell tissue culture test. Similar examination of VT⁺ O 157 strains isolated in North America showed that they were not invasive (Wells *et al.* 1983).

DISCUSSION

In a preliminary communication we have described the production of two Vero cytotoxins by strains of *E. coli* O 157. This was based on serum neutralization experiments and the properties of bacteriophages carrying VT genes isolated from these strains (Scotland, Smith & Rowe, 1985). Subsequently specific DNA probes for both VT 1 and VT 2 have been developed from these phages (Willshaw *et al.* 1985, 1987). By hybridization tests we have identified three classes of VT⁺ O 157 strains which hybridized with either the VT 1 probe, or the VT 2 probe, or with both probes. Serum neutralization tests confirmed the production of one or two toxins in agreement with the probe results, and we conclude that both toxins are virulence factors. Neutralization of the toxins produced by VT1⁺/VT2⁺ strains was not observed in any experiment using anti-Shiga toxin (VT 1) alone; for some tests the titres of VT 1 and VT 2 were similar and for other tests VT 2 titres were higher than those of VT 1. The strains from North America produced both VT 1 and VT 2. Strains producing only VT 2 were common amongst the strains isolated in the United Kingdom, particularly from cases of HUS. In our collection of over 70 VT⁺ strains of O 157 isolated in the United Kingdom there is only one strain producing VT 1 and not VT 2 (unpublished results).

All VT⁺ strains carried a plasmid with a molecular weight between 55×10^6 and 63×10^6 . This plasmid has been associated with the ability to attach to epithelial cells (Karch *et al.* 1987). For some strains this was the only plasmid carried, but usually other plasmids were present. The majority of strains producing VT 1 and VT 2 also produced colicin D and carried a plasmid with a molecular weight of $c. 4.7 \times 10^6$. Strains producing VT 2 only did not have a plasmid of this size. Further experiments showed that this plasmid encoded colicin D production (Willshaw, unpublished results). There were a number of different plasmid profiles, and these have been useful in identifying strains related to a particular outbreak.

Strains which did not produce VT in the tissue culture test did not hybridize with either VT probe. None of the VT⁻ strains of human or animal origin was H type 7. Eighteen of the 21 VT⁻ strains of human origin were H45. The plasmid profiles of the VT⁺ strains were relatively simple, but the profiles of the VT⁻ strains were more complex (Table 2). Certain plasmids probably encoded antibiotic resistance or colicin production and, for the porcine strains, also K 88 fimbriae and toxin production. No two VT⁻ strains of human origin had common plasmid profiles although the majority were of the same serotype, O157.H45. All the strains of this serotype carried a large plasmid, but the functions encoded on this plasmid are not known. Although strains of serogroup O157 are not a common cause of extra-intestinal infections, most of these human VT⁻ strains were isolated from blood, the eye or urine and it is probable that they do represent a group with special pathogenic abilities. Strain SP88 of serotype O157.H45 isolated from urine possesses fimbriae which belong to the fimbrial antigen group F16, which has been associated with uropathogenic strains (Parry & Rooke, 1985; Ørskov, personal communication).

Strains of *E. coli* O157 belong to at least three pathogenic groups. First, strains of H type 45, whose virulence mechanisms are not known, appear to be associated with extra-intestinal infections in humans. Secondly, strains producing enterotoxins and possessing the adhesive factor K 88 cause enteric colibacillosis in pigs. Although VT⁺ strains have been isolated from animals with enteric disease, to our knowledge these have not belonged to serogroup O157. Thirdly, strains producing Vero cytotoxin and almost invariably of H type 7 cause diarrhoea in humans, which is usually bloody. VT⁺ O157 strains have also been associated with HUS, which may be preceded by bloody diarrhoea. Beef products and milk have been implicated as the vehicles of infection in several outbreaks of HC (Riley *et al.* 1983; Ryan *et al.* 1986), and although VT⁺ O157 strains have not been shown to be a cause of animal disease, they have been isolated from milk and healthy cattle (Martin *et al.* 1986; Borczyk *et al.* 1987), indicating the zoonotic nature of the disease. Although the importance of VT⁺ O157 strains as a cause of human disease is established, there is a need to identify the modes of action of both VT1 and VT2 and to study the stages of pathogenesis in which they are involved, including the immunological response.

REFERENCES

- ANDERSON, E. S. & THRELFALL, E. J. (1974). The characterization of plasmids in the enterobacteria. *Journal of Hygiene* **72**, 471-487.
- BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513-1523.
- BORCZYK, A. A., KARMALI, M. A., LIOR, H. & DUNCAN, L. M. C. (1987). Bovine reservoir for Verotoxin-producing *Escherichia coli* O157.H7. *Lancet* *i*, 98.
- DAY, N. P., SCOTLAND, S. M., CHEASTY, T. & ROWE, B. (1983). *Escherichia coli* O157.H7 associated with human infections in the United Kingdom. *Lancet* *i*, 825.
- DEAN, A. G., CHING, Y. C., WILLIAMS, R. G. & HARDEN, L. B. (1972). Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *Journal of Infectious Diseases* **125**, 407-411.
- FARMER, J. J. & DAVIS, B. R. (1985). H7 antiserum-sorbitol fermentation medium for detecting *Escherichia coli* O157.H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology* **22**, 620-625.

- FUROWICZ, A. J. & ØRSKOV, F. (1972). Two new *Escherichia coli* O antigens, O 150 and O 157, and one new K antigen, K 93, in strains isolated from veterinary disease. *Acta Pathologica Microbiologica Scandinavica*, Section B **80**, 441-444.
- GRANDSEN, W. R., DAMM, M. A. S., ANDERSON, J. D., CARTER, J. E. & LIOR, H. (1986). Further evidence associating haemolytic uraemic syndrome with infection by Verotoxin-producing *Escherichia coli* O 157.H7. *Journal of Infectious Diseases* **154**, 522-524.
- GROSS, R. J. & ROWE, B. (1985). Serotyping of *Escherichia coli*. In *The Virulence of Escherichia coli. Reviews and Methods* (ed. M. Sussman), pp. 345-363. London: Academic Press.
- JOHNSON, W. M., LIOR, H. & BEZANSON, G. S. (1983). Cytotoxic *Escherichia coli* O 157H7 associated with haemorrhagic colitis in Canada. *Lancet* **i**, 76.
- KARCH, H., HEESEMAN, J., LAUFS, R., O'BRIEN, A. D., TACKET, C. O. & LEVINE, M. M. (1987). A plasmid of enterohemorrhagic *Escherichia coli* O 157.H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. *Infection and Immunity* **55**, 455-461.
- KARMALI, M. A., PETRIC, M., LIM, C., FLEMING, P. C., ARBUS, G. S. & LIOR, H. (1985). The association between idiopathic hemolytic uremic syndrome and infection by Verotoxin-producing *Escherichia coli*. *Journal of Infectious Diseases* **151**, 775-782.
- KONOWALCHUK, J., SPEIRS, J. I. & STAVRIC, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. *Infection and Immunity* **18**, 775-779.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MARCH, S. B. & RATNAM, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* associated with hemorrhagic colitis. *Journal of Clinical Microbiology* **23**, 869-872.
- MARTIN, M. L., SHIPMAN, L. D., WELLS, J. G., POTTER, M. E., HEDBERG, K., WACHSMUTH, I. K., TAUXE, R. V., DAVIS, J. P., ARNOLDI, J. & TILLELI, J. (1986). Isolation of *Escherichia coli* O 157.H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. *Lancet* **ii**, 1043.
- MORRIS, J. A., & SOJKA, W. J. (1985). *Escherichia coli* as a pathogen in animals. In *The Virulence of Escherichia coli: Reviews and Methods* (ed. M. Sussman) pp. 47-77. London: Academic Press.
- NEILL, M. A., AGOSTI, J. & ROSEN, H. (1985). Hemorrhagic colitis with *Escherichia coli* O 157.H7 preceding adult hemolytic uremic syndrome. *Archives of Internal Medicine* **145**, 2215-2217.
- NEWLAND, J. W., STROCKBINE, N. A., MILLER, S. F., O'BRIEN, A. D. & HOLMES, R. K. (1985). Cloning of Shiga-like structural genes from a toxin converting phage of *Escherichia coli*. *Science* **230**, 179-181.
- O'BRIEN, A. D. & LAVECK, G. D. (1983). Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. *Infection and Immunity* **40**, 675-683.
- O'BRIEN, A. D., LIVELY, T. A., CHEN, M. E., ROTHMAN, S. W. & FORMAL, S. B. (1983). *Escherichia coli* O 157.H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (Shiga)-like cytotoxin. *Lancet* **i**, 702.
- O'BRIEN, A. D., NEWLAND, J. W., MILLER, S. F., HOLMES, R. K., SMITH, H. W. & FORMAL, S. B. (1984). Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* **226**, 694-696.
- PAI, C. H., GORDON, R., SIMS, H. V. & BRYAN, L. E. (1984). Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O 157.H7. Clinical, epidemiologic, and bacteriologic features. *Annals of Internal Medicine* **101**, 738-742.
- PALMER, S. R. (1986). A community outbreak of haemorrhagic colitis due to verotoxin producing *Escherichia coli* O 157 H7. *Proceedings of the 2nd World Congress. Foodborne Infections and Intoxications*. Berlin: Institute of Veterinary Medicine - Robert von Ostertag Institute.
- PARRY, S. H. & ROOKE, D. M. (1985). Adhesins and colonization factors of *Escherichia coli*. In *The Virulence of Escherichia coli. Reviews and Methods* (ed. M. Sussman), pp. 79-155. London: Academic Press.
- PUGSLEY, A. P. (1985). *Escherichia coli* K12 strains for use in the identification and characterization of colicins. *Journal of General Microbiology* **131**, 369-376.
- RATNAM, S. & MARCH, S. B. (1986). Sporadic occurrence of hemorrhagic colitis associated with *Escherichia coli* O 157.H7 in Newfoundland. *Canadian Medical Association Journal* **134**, 43-49.

- REMIS, R. S., MACDONALD, K. L., RILEY, L. W., PUHR, N., WELLS, J. G., DAVIS, B. R., HEBERT, R. J., OLCOTT, E. S., JOHNSON, L. M., HARGRETT, N. T., BLAKE, P. A. & COHEN, M. L. (1984). Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157. H7. *Annals of Internal Medicine* **101**, 624-626.
- RILEY, L. W., REMIS, R. S., HELGERSON, S. D., MCGEE, H. B., WELLS, J. G., DAVIS, B. R., HEBERT, R. J., OLCOTT, E. S., JOHNSON, L. M., HARGRETT, N. T., BLAKE, P. A. & COHEN, M. L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* **308**, 681-685.
- RYAN, C. A., TAUXE, R. V., HOSEK, G. W., WELLS, J. G., STOESZ, P. A., MCFADDEN, H. W., SMITH, P. W., WRIGHT, G. F. & BLAKE, P. A. (1986). *Escherichia coli* O157. H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. *Journal of Infectious Diseases* **154**, 631-638.
- SCOTLAND, S. M., DAY, N. P. & ROWE, B. (1980). Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. *FEMS Microbiology Letters* **7**, 15-17.
- SCOTLAND, S. M., GROSS, R. J. & ROWE, B. (1985). Laboratory tests for enterotoxin production, enteroinvasion and adhesion in diarrhoeagenic *Escherichia coli*. In *The Virulence of Escherichia coli. Reviews and Methods* (ed. M. Sussman), pp. 395-405. London: Academic Press.
- SCOTLAND, S. M., ROWE, B., SMITH, H. R., WILLSHAW, G. A. & GROSS, R. J. (1987). Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. *Journal of Medical Microbiology*. In press.
- SCOTLAND, S. M., SMITH, H. R. & ROWE, B. (1985). Two distinct toxins active on Vero cells from *Escherichia coli* O157. *Lancet* **ii**, 885-886.
- SMITH, H. R., DAY, N. P., SCOTLAND, S. M., GROSS, R. J. & ROWE, B. (1984). Phage-determined production of Vero cytotoxin in strains of *Escherichia coli* serogroup O157. *Lancet* **i**, 1242-1243.
- SMITH, H. R., ROWE, B., GROSS, R. J., FRY, N. K. & SCOTLAND, S. M. (1987). Haemorrhagic colitis and Vero cytotoxin-producing *Escherichia coli* in England and Wales. *Lancet* **i**, 1062-1065.
- SPIKA, J. S., PARSONS, J. E., NORDENBERG, D., WELLS, J. G., GUNN, R. A. & BLAKE, P. A. (1986). Hemolytic uraemic syndrome and diarrhea associated with *Escherichia coli* O157. H7 in a day care center. *Journal of Pediatrics* **109**, 287-291.
- STROCKBINE, N., MARQUES, L. R. M., NEWLAND, J. W., SMITH, H. W., HOLMES, R. K. & O'BRIEN, A. D. (1986). Two toxin-converting phages from *Escherichia coli* O157. H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infection and Immunity* **53**, 135-140.
- SWEENEY, E. J. (1970). Strains of *Escherichia coli* associated with mortality in pigs. *Irish Veterinary Journal* **24**, 108-113.
- TAYLOR, C. M., WHITE, R. H. R., WINTERBORN, M. H. & ROWE, B. (1986). Haemolytic-uraemic syndrome: clinical experience of an outbreak in the West Midlands. *British Medical Journal* **292**, 1513-1516.
- WELLS, J. G., DAVIS, B. R., WACHSMUTH, I. K., RILEY, L. W., REMIS, R. S., SOKOLOW, R. & MORRIS, G. K. (1983). Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *Journal of Clinical Microbiology* **18**, 512-520.
- WILLSHAW, G. A., SMITH, H. R. & ANDERSON, E. S. (1979). Application of agarose gel electrophoresis to the characterization of plasmid DNA in drug-resistant enterobacteria. *Journal of General Microbiology* **114**, 15-25.
- WILLSHAW, G. A., SMITH, H. R., SCOTLAND, S. M., FIELD, A. M. & ROWE, B. (1987). Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. *Journal of General Microbiology* **133**, 1309-1317.
- WILLSHAW, G. A., SMITH, H. R., SCOTLAND, S. M. & ROWE, B. (1985). Cloning of genes determining the production of Vero cytotoxin by *Escherichia coli*. *Journal of General Microbiology* **131**, 3047-3053.
- WILSON, R. A. & FRANCIS, D. H. (1986). Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. *American Journal of Veterinary Research* **47**, 213-217.