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Phage-typing of Vero-cytotoxin (VT) producing *Escherichia coli* O157 isolated in the United Kingdom

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SUMMARY

Vero-cytotoxin (VT) producing *Escherichia coli* serogroup O157 have been isolated from patients with diarrhoea, haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). A phage-typing scheme developed in Canada has been used to type 155 VT^+ *E. coli* O157 serogroup isolated from sporadic infections in the UK since 1983, and 48 strains from HC or HUS outbreaks. Twelve phage types were identified of which three, types 49, 51 and 52, have not been found in North America. All strains carried a 60×10^6 plasmid and most $VT1^+VT2^+$ strains also had a 5×10^6 plasmid coding for colicin D production. The majority of strains producing both VT1 and VT2 belonged to phage type 1, or the related types 4, 8 and 14. Most strains producing only VT2 belonged to types 2 or 49. Four outbreaks were included in the survey. Three had strains of a single phage type while strains from the fourth outbreak were more variable. The distribution of phage types throughout the UK showed no marked geographical variations.

INTRODUCTION

Strains of *Escherichia coli* which produce a heat labile cytotoxin active on Vero cells, termed Vero-cytotoxin (VT) (1), have been shown to be associated with outbreaks and sporadic cases of haemorrhagic colitis (HC) (2,3) and haemolytic uraemic syndrome (HUS) (4,5). Neutralization experiments have shown that there are at least two distinct Vero-cytotoxins (6), VT1 and VT2, and that VT production is most frequently associated with *E. coli* belonging to serogroup O157 which either possess flagellar antigen H7 or are non-motile. As well as sporadic cases of HUS and HC throughout England and Wales, there have been several outbreaks associated with serotype O157:H7 usually occurring in the summer months between April and October. These include outbreaks of HUS in the West Midlands in 1983 (5,7) and of HC in East Anglia in 1985 (3,8) and Birmingham in 1987 (9). There was also a small outbreak among travellers returning from an hotel in Ibiza in 1986 (3).

To assist in the epidemiological investigation of infections caused by VT producing *E. coli* (VTEC) a phage-typing scheme for VT^+ *E. coli* O157 has been developed in Canada (10). The scheme uses 16 phages which originally defined 14

phage types. In Canada, the results demonstrated good correlation with epidemiological information in that strains from the same outbreak, or from a patient and incriminated food, belonged to the same phage type (10, 11).

Further use of the scheme in Canada and the UK has added another 38 types (H. Lior, personal communication).

VTEC belonging to serogroup O157 have been isolated in the UK from cases of HC and HUS, and non-bloody diarrhoea (12). These strains have been phage typed and the results correlated with production of VT, hybridization with DNA probes for VT1 and VT2, colicin production, and plasmid profiles.

MATERIALS AND METHODS

Bacterial strains

The strains were from the Division of Enteric Pathogens' collection of *E. coli* O157 isolated in the UK between 1981 and August 1988. A single colony from each strain had been serotyped (13) using antisera for *E. coli* somatic (O) antigens 1–170, and flagellar (H) antigens 1–56 and stored on Dorset egg agar medium at room temperature.

The sample included 98 strains from sporadic HC cases, 13 from HUS and 44 from patients with diarrhoea. Forty-eight strains from 1 HUS outbreak and 3 HC outbreaks described previously (3, 5, 9, 12) were also included.

Phage typing

Phage stocks and control strains for the phage-typing scheme developed in Canada (10) were kindly supplied by H. Lior, Ottawa, Canada. There are 16 phages and 52 phage types have been described to date (H. Lior, personal communication). Cultures for phage-typing were grown in nutrient broth for 18 h at 37 °C then 0·1 ml inoculated into 3 ml of nutrient broth and grown for 1–2 h at 37 °C with aeration. Plates were inoculated by flooding with 2 ml of the fresh culture and, when dry, 0·01 ml samples of phage at routine test dilution (RTD) were applied. Plates were read after overnight incubation at 37 °C.

When first tested several strains gave patterns intermediate between two types. These strains were plated and single colonies were phage-typed and rechecked for variations in other properties.

Vero cytotoxin production

VT production was tested as described previously (14) using sterile culture supernatants after growth in trypticase soy broth with dextrose (BBL).

Colicinogeny

Strains were tested for colicinogeny by overlaying with a colicin sensitive *E. coli*. Colicins were identified by overlaying with colicin-resistant or colicin-insensitive strains as described by Pugsley (15).

DNA hybridization

VT1 and VT2 were identified using probes derived from phage genes cloned into recombinant plasmids NTP705 for VT1 (16) and NTP707 for VT2 (17). Strains

were also tested for hybridization with the 3·4 kb EHEC (enterohaemorrhagic *E. coli*) probe CVD419 developed by Levine and others from the 60×10^6 plasmid in strain 933 (18). Broth cultures were spotted onto nylon filters (Hybond-N, Amersham) on MacConkey agar plates and incubated at 37 °C for 5–6 h. Filters were prepared for hybridization as described by Maniatis, Fritsch & Sambrook (19). Hybridization and washing at high stringency was as described by Willshaw and others (16).

Plasmid characterization

Plasmid DNA was prepared by the method of Birnboim & Doly (20) and visualized by agarose gel electrophoresis (21).

RESULTS AND DISCUSSION

VT production was confirmed in all of the strains in the present survey. All hybridized with one or both of the VT probes, and with the CVD419 probe. All carried a plasmid of molecular weight between 51×10^6 and 63×10^6 (12) (Table 1). A plasmid of this size has also been reported in VT^+ strains from Canada (22) and the USA (23), and it has been reported that it encodes a fimbrial antigen that is involved in adhesion (24). The plasmid isolated from strain E29962 is typical of the 60×10^6 plasmids and has been designated pDEP18.

Strains from sporadic infections

Of the 155 VT^+ strains of *E. coli* O157 from sporadic infections, 140 were O157 : H7 and 15 O157 : H −. Fifty-three strains carried a small plasmid of $4–5 \times 10^6$ and 45 of these produced colicin D. A plasmid of *c.* 5×10^6 has been shown to code for colicin D production in some of these strains (12). This plasmid was usually associated with strains producing both VT1 and VT2 and colicin D. The other plasmids present in 54 strains, ranging in size from 1×10^6 to 98×10^6, have not been shown to correlate with VT production, colicinogeny or phage type.

Relationship between phage type and VT type

All of the VT^+ strains examined were typable with the phages described by Ahmed and others (10). Of the 155 strains from sporadic infections, 57 hybridized with both VT1 and VT2 probes, 3 with VT1 only and 95 with VT2 only. The two most common phage types were type 1 (38 strains, 24·5 % of total) and type 2 (49 strains, 31·6 %) (Table 2).

(*a*) *Phage types 1, 4, 8 and 14*

These types are related in that they differ only in reactions with 4 of the 16 phages, phages 6, 9, 10 and 13 (10) and usually produce VT1, VT2 and colicin D (Table 3). Some strains gave intermediate readings when phage-typed and single colony picks were found to belong to different types indicating a degree of instability. The most variable phage type was type 1 which gave rise to colonies of types 4, 8, and 14. The changes were unidirectional and due to increasing sensitivity to the typing phages. Type 1 is resistant to phages 6, 9, 10 and 13, type 4 is sensitive to phages 6 and 13, type 8 is sensitive to phages 9 and 10 and type

Table 1. *Phage typing of 155* E. coli *O157 VT⁺ strains from sporadic infections*

Phage type	Total	VT1	VT2	Colicin	Plasmids – molecular size range × 10^6					Number of strains O157:H7	Number of strains O157:H–
1	38	+	+	D	51–63			4–5		31*	
		+	+	—	56–61			5		5	
		–	+	D	55	38		5		1	
		+	+	Untypable	59			4	3	1	
2	49	–	+	—	53–63					24†	
		–	+	—	51–65	35–45				23‡	
		–	+	I	56–57					1	
		–	+	E2	59		25			1	
4	14	+	+	D	54–60			4–5		3	
		–	+	D	54			5		1	
		–	+	—	56–64					6	1
		+	+	—	56–60		27			2	
		+	+	Untypable	59			4		1	
8	6	+	+	—	57			4		1	
		+	+	—	61		24	5		1	
		+	+	—	60					0	1
		+	+	—	58	37				0	1
		+	–	D	52–59			4–5		2	
14	8	–	+	—	56			3		1	
		+	+	—	55–64	38–42				2	1
		+	+	—	59					0	2
		+	+	—	53			4		1	
		+	–	D	58			4		1	
21	2	+	+	—	53					1	
		+	+	—	98 57					1	

24	3	−	+	I	53–58					1	1		1
		+	+	D	59	57		5		1			
32	8	−	+	—	52–63					3			
		−	+	—	56–60				1–2	4			
		−	+	D	61			5	2	1			
43	1	+	+	Untypable	58	42				1			
49	22	−	+	—	52–63					9	3		
		−	+	—	55–57		21–22			1	2		
		−	+	—	55–56	35–42				2§	1		
		−	+	—	58				3	1			
		−	+	I	55					0	1		
		−	+	I	50–66	44–50				1	1		
50	2	−	+	—	60		26			1			
		−	+	I	55	38	26			1			
51	2	−	+	—		57			2	2			

Production of VT1 and VT2 was ascertained by hybridization with appropriate probes.
* Eight strains each carried one additional plasmid ranging in size from $3–68 \times 10^6$.
† Two strains carried an additional plasmid between 16×10^6 and 23×10^6.
‡ One strain carried a plasmid of 5×10^6 and two strains each had two plasmids in the range $35–40 \times 10^6$.
§ One strain had two plasmids in the range $37–42 \times 10^6$.

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Table 2. *Correlation of VT production with phage type*

VT Production	Phage type												Total
	1	2	4	8	14	21	24	32	43	49	50	51	
VT1 VT2	37		6	4	6	2	1		1				57
VT1				2	1								3
VT2	1	49	8		1		2	8		22	2	2	95
No. of strains	38	49	14	6	8	2	3	8	1	22	2	2	155

Table 3. *Phage type changes in types 1, 4, 8 and 14*

Phage type	VT1	VT2	Sensitivity to typing phage 6	9	10	13
1	+	+	−	−	−	−
4	+	+	−	+	+	−
8	+	−	+	−	−	+
14	+	−	+	+	+	+

14 is sensitive to all 4 phages. The conversion from type 1 to 8, or 4 to 14 was invariably accompanied by loss of hybridization with the VT2 probe suggesting that some VT 2 phages restrict the typing phages and are phage type determining. There was no change in plasmid content consequent to these phage type changes. The change from type 1 to type 4, was however not accompanied by changes in VT production nor by plasmid loss. Loss of VT1 production was observed in several strains of phage types 1 or 4 but was not correlated with any change in phage type.

Fourteen strains belonged to phage type 4. Three of these resembled type 1 strains in their VT and colicin characteristics. A further seven strains produced VT2 only, were non-colicinogenic and carried only one plasmid. Two strains, from related patients, produced VT1 and VT2 but were not colicin D producers. Of the remaining two strains of phage type 4, one produced VT2 and colicin D, and the other VT1, VT2 and an untypable colicin.

Strains assigned to phage type 8 could be divided into two groups. Two strains produced VT1 and colicin D. These were probably derived from phage type 1 following loss of VT2 genes. The remaining four strains consistently produced VT1 and VT2, but were non-colicinogenic although two carried a plasmid of approximately 5×10^6.

Phage type 14 is sensitive to all the typing phages. Of the 8 strains from infections acquired in the UK, 6 produced VT1 and VT2 and were non-colicinogenic; 3 of these carried only the pDEP18-like plasmid and 3 also had a $38–42 \times 10^6$ plasmid. One type 14 strain produced VT2 only and one VT1 and colicin D. The latter may have been derived from type 4 following loss of VT2 as described above.

(*b*) *Phage types 2 and 24*

All 49 strains of phage type 2 produced VT2 only; only two were colicinogenic and these produced colicin I or colicin E2. All had a pDEP18-like plasmid but none had the 5×10^6 plasmid associated with colicin D production.

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Table 4. *Phage typing of 48* E. coli *O157 VT*$^+$ *strains from outbreaks*

Location	No. of strains tested	Date	Strain characteristics: Phage type	VT1	VT2	Colicin	Plasmids (MW × 10^6)			No. of strains
West Midlands	2	1983	2	–	+	I	56			1
(HUS)			2	–	+	—	59			1
East Anglia	24	1985	1	+	+	D	51–63		4–5	17
(HC)			2	–	+	—	57	16		1
			4	+	+	D	57–59		4	3
			4	+	+	D	57–59	27	4	2
			24	+	+	D	59 51		5	1
Ex Ibiza (HC)	6	1986	14	–	+	—	55–57	38–39		6
West Midlands (HC)	16	1987	2	–	+	—	54–60			16

Two of the three strains of phage type 24 produced VT2 and colicin I. These are related to phage type 2 in that transfer of the colI plasmid from a type 24 strain converted type 2 to type 24. Conversely, loss of the colI plasmid from a type 24 strain resulted in a change to phage type 2. The colicin plasmid is masked in the plasmid profile as it is the same size (approximately 60×10^6) as the pDEP18-like plasmid present in all VT$^+$ O157 strains. The other sporadic isolation appears to be more closely related to phage type 1 since it produced both VT1, VT2 and colicin D.

(*c*) *Other phage types*

Two strains of phage type 21 were identified. These produced VT1 and VT2 and were non-colicinogenic. One strain had only the pDEP18-like plasmid, while the second had an additional plasmid of 98×10^6. Segregants of these strains which had lost VT2 converted to phage type 32. Types 21 and 32 differ only in reactions with phages 6 and 13, the same phages as were involved in the change from type 1 to type 8. There were eight strains of phage type 32 all of which produced VT2 only.

One strain of phage type 43 was identified. This was a VT1 and VT2 producer and also produced an untypable colicin.

The remaining 26 strains had patterns of phage reactions which had not previously been described for strains isolated in Canada. Type strains for these were sent to Canada and they have been assigned type designations 49, 50 and 51 in accordance with the current Canadian scheme (H. Lior, personal communication). Type 49 accounted for 22 of these and all strains produced VT2 only; three also produced colicin I. All carried the pDEP18-like plasmid and eight had second plasmids (see Table 1). Eight of the type 49 strains were non-motile. There were two strains each of types 50 and 51.

Strains from outbreaks

Of the 24 strains isolated during the HC outbreak in East Anglia in 1985, 17 were phage type 1 and 5 phage type 4 (Table 4). These two types are related as

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described above. One strain each of types 2 and 24 were also isolated. The type 24 strain produced both VT1 and VT2, and colicin D and may be related to the outbreak strain whereas the type 2 strain was non-colicinogenic and produced VT2 only. However, since the outbreak occurred over a wide geographical area the type 2 strain was probably a sporadic infection not related to the outbreak.

Strains from a small HC outbreak among holidaymakers returning from Ibiza (3) produced VT2 only (Table 4) and belonged to phage type 14. This pattern of phage type, plasmid profile and VT production has not been seen among strains originating in the UK.

All of the strains from both of the West Midlands outbreaks were phage type 2 (Table 4) although these outbreaks were separated by several years.

Geographical distribution

Within each of the four outbreaks which occurred between 1983 and 1987 all strains isolated belonged to the same, or related, phage types and had similar patterns of VT production and plasmid profile with the exception of the phage type 2 strain from East Anglia.

The 155 strains from sporadic infections occurred throughout the country with largest numbers from the North East (34 strains), East Anglia (32), Scotland (22) and the West Midlands (22). There were no clear geographical variations in phage type distribution except for a possibly higher proportion of phage type 2 strains in East Anglia (14 of 32 strains).

Phage type 49, one of the 'new' types has been isolated throughout the UK, and was the second most common type isolated in the first 8 months of 1988 (12 of 45 strains, 26·7%). Of the phage types now described in Canada (H. Lior, personal communication) only nine have been identified in the UK survey. The isolation frequencies of different phage types vary between the two countries. Phage type 2 was the most common type in the present UK survey (34% of strains tested), followed by type 1 (27%). In a similar survey (11) of 174 strains isolated in North America the same phage types, 1 and 2 predominated with 43% of strains belonging to type 1 and 22% to type 2. Within each phage type, the most common plasmid profiles were similar in both UK and North American strains (11).

This survey demonstrates the value of the phage typing scheme. More precise epidemiological information can be obtained if phage typing is used in conjunction with VT characterization and plasmid profile analysis.

REFERENCES

1. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977; **18**: 775–9.
2. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Eng J Med 1983; **308**: 681–5.
3. Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and Vero cytotoxin-producing *Escherichia coli* in England and Wales. Lancet 1987; i: 1062–5.
4. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983; i: 619–20.

5. Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol 1988; **25**: 237–43.
6. Scotland SM, Smith HR, Rowe B. Two distinct toxins active on Vero cells from *Escherichia coli* O157. 1985; Lancet; ii: 885–6.
7. Taylor CM, White RHR, Winterborn MH, Rowe B. Haemolytic-uraemic syndrome: clinical experience of an outbreak in the West Midlands. Br Med J 1986; **292**: 1513–6.
8. Morgan GM, Newman C, Palmer SR, et al. First recorded community outbreak of haemorrhagic colitis due to Verotoxin-producing *Escherichia coli* O157.H7 in the UK. Epidemiol Infect 1988; **101**: 83–91.
9. *Communicable Disease Report* 1987; 87/26.
10. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage-typing scheme for *Escherichia coli* O157:H7. J Infect Dis 1987; **155**: 806–9.
11. Ratnam S, March SB, Ahmed R, Bezanson GS, Kasatiya S. Characterization of *Escherichia coli* serotype O157:H7. J Clin Microbiol 1988; **26**: 2006–12.
12. Scotland SM, Willshaw GA, Smith HR, Rowe B. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. Epidemiol Infect 1987; **99**: 613–24.
13. Gross RJ, Rowe B. Serotyping of *Escherichia coli*. In: Sussman M, ed. The virulence of *Escherichia coli*. Reviews and Methods. London: Academic Press, 1985; 345–63.
14. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Microbiol Lett 1980; **7**: 15–17.
15. Pugsley AP. *Escherichia coli* K12 strains for use in the identification and characterization of colicins. J Gen Microbiol 1985; **131**: 369–76.
16. Willshaw GA, Smith HR, Scotland SM, Rowe B. Cloning of genes determining the production of Vero cytotoxin by *Escherichia coli*. J Gen Microbiol. 1985; **131**: 3047–53.
17. Willshaw GA, Smith HR, Scotland SM, Field AM, Rowe B. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J Gen Microbiol 1987; **133**: 1309–17.
18. Levine MM, Xu J-G, Kaper JB, et al. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1987; **156**: 175–82.
19. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
20. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl Acid Res 1979; **7**: 1513–1523.
21. Willshaw GA, Smith HR, Anderson ES. Application of agarose gel electrophoresis to the characterization of plasmid DNA in drug-resistant enterobacteria. J Gen Microbiol 1979; **114**: 15–25.
22. Johnson WM, Lior H, Bezanson GS. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. Lancet 1983; i: 76.
23. Wells JG, Davis BR, Wachsmuth IK, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J Clin Microbiol 1983; **18**: 512–20.
24. Karch H, Heesemann J, Laufs R, O'Brien AD, Tacket CO, Levine MM. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect Immun 1987; **55**: 455–61.