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Virulence properties of *Escherichia coli* strains belonging to serogroups O26, O55, O111 and O128 isolated in the United Kingdom in 1991 from patients with diarrhoea

S. M. SCOTLAND, G. A. WILLSHAW, H. R. SMITH, B. SAID, N. STOKES AND B. ROWE

Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

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SUMMARY

Some strains of *Escherichia coli* belonging to serogroups O26, O55, O111 or O128 produce Vero cytotoxin (VT). These serogroups are included in the range of enteropathogenic *E. coli* (EPEC) serogroups for which commercial antisera are available. In an attempt to obtain information on VT-producing strains other than those of serogroup O157, 122 strains belonging to these four serogroups and isolated in 1991 from patients with diarrhoea in the United Kingdom were tested for hybridization with VT probes. Only 18 of the 122 strains were VT-positive and these were O26 or O128. However 90 strains hybridized with the *E. coli* attaching and effacing (*eae*) probe (including 14 VT-positive strains) and 17 with the enteroaggregative *E. coli* (EAggEC) probe. For 78 *eae*-positive and 9 EAggEC-positive strains, tissue culture tests correlated with the probe results as the strains gave, respectively, either localized adhesion and a positive fluorescent-actin staining test or a characteristic aggregative attachment. A total of 111 of the 122 strains belonging to serogroups O26, O55, O111 or O128 possessed properties that may be associated with the ability to cause human diarrhoeal disease, and similar studies are needed on strains from the other classical EPEC serogroups.

INTRODUCTION

Strains of *Escherichia coli* producing Vero cytotoxin (VTEC) are now recognized as a cause of disease in man ranging from mild diarrhoea to haemorrhagic colitis (HC) and the haemolytic uraemic syndrome (HUS) [1, 2]. In particular VTEC of serogroup O157 have been isolated from many outbreaks and sporadic cases of HC in North America and the United Kingdom [3, 4]. O157 VTEC differ in several biochemical properties from most *E. coli* strains; for example they usually fail to ferment sorbitol promptly and they lack β-glucuronidase activity. These differences have been utilized in the development of media, such as sorbitol MacConkey agar, that can be used to assist in the isolation of O157 VTEC. However, VTEC belonging to many other O groups have been isolated from cases of diarrhoea or HUS, and as yet these have not been shown to possess any discriminatory characteristic other than VT production. To recognize the presence of such VTEC in faecal samples, colonies have to be tested directly for VT

production or for hybridization with VT gene probes. These techniques are not available in most routine laboratories and so the incidence of VTEC belonging to serogroups other than O157 has been poorly studied.

The serogroups to which VTEC may belong include O26, O55, O111 and O128 [5–11]. These are included in the range of O groups of enteropathogenic *E. coli* (EPEC) and antisera are available commercially for their identification. Such antisera remain in use in many clinical laboratories and so in January 1991 the Laboratory of Enteric Pathogens requested recent isolates of *E. coli* that belonged to these four serogroups from cases of diarrhoea, haemorrhagic colitis or haemolytic uraemic syndrome (HUS). The strains received have been tested for hybridization with DNA probes for the VT1 and VT2 genes. They were also tested for hybridization with several virulence-related DNA probes, including the *eae* probe that encodes sequences necessary for the ability to cause attaching and effacing lesions in the intestine. In addition they were examined for adhesion to HEp-2 cells; this can be characterized as localized, aggregative or diffuse and has been used to define the enteroaggregative *E. coli* (EAggEC) or diffusely-adherent *E. coli* (DAEC).

METHODS AND MATERIALS

Bacterial strains

The 122 *E. coli* strains included in the study were received from 27 laboratories in many different areas of the United Kingdom. They had been isolated from the faeces of patients with diarrhoea during 1991. There were no cases of HUS. Except for one small family outbreak the strains were from sporadic cases. Information was requested, but not always given, on whether the diarrhoea had been bloody and about the duration of the diarrhoea. The strains were serotyped using standard agglutination methods [12].

DNA hybridization

Strains were tested by colony hybridization. The VT1 probe was a 0·75 kb *Hinc* II fragment derived from strain H19 (*E. coli* O26:H11) and the VT2 probe was a 0·85 kb *Ava* I-*Pst* I fragment derived from E32511 (*E. coli* O157:H−) [13]. The VT probes were labelled with digoxigenin and used as described previously [14]. The CVD419 probe was a 3·4 kb *Hin*d III fragment derived from a plasmid in strain 933 (*E. coli* O157:H7) [15]. The *eae* (*E. coli* attaching and effacing) [16] and EAF (enteropathogenic *E. coli* adherence factor) [17] probes were derived respectively from chromosomal and plasmid genes of E2348/69 (*E. coli* O127:H6). The EAggEC probe was that described by Baudry and coworkers [18] and the DAEC probe was a 370 bp *Pst* I fragment within the *daaC* gene of strain C1845 [19]. Probes were labelled with deoxyadenosine-5′-α-thio[^{35}S]-triphosphate [13]. Hybridization and washing were under stringent conditions [20] except for the EAggEC probe that was used as described by Moseley and colleagues [21].

Cell culture adhesion tests

Adhesion to HEp-2 cells was tested in the presence of D-mannose (1% w/v) as described previously [22]. Adhesion was assessed as localized, diffuse or aggregative at the end of a 6 h test during which the monolayer was washed after

an initial 3 h attachment period. The fluorescence actin staining (FAS) test, which demonstrates the presence of filamentous actin beneath attached bacteria, was that of Knutton and colleagues [23]. At least two tests were performed before a strain was recorded as not giving attachment.

Tests for enterohaemolysin and α-haemolysin

Strains were tested on blood agar plates as described previously [10]. They were considered positive for enterohaemolysin (E-Hly) when they lysed washed sheep erythrocytes but not unwashed horse erythrocytes. Strains producing α-haemolysin (α-Hly) were lytic on both media and were confirmed as α-haemolytic by a colony immunoblot assay [24] using a specific antiserum kindly supplied by Dr T. J. Baldwin.

RESULTS

One hundred and twenty-two strains belonging to O groups 26, 55, 111 or 128 were tested for hybridization with probes for VT1 or VT2 (Table 1–4). Only 18 strains were positive and these were either O26 or O128. Fourteen O26 VTEC were all of H type 11 and four O128 VTEC were H2 or non-motile. The VT type of the strains is given in the tables. However, 93 of the 104 VT-negative strains were positive in other tests and details of these investigations are given below for each serogroup.

Strains of E. coli *O26*

Thirty-nine strains of *E. coli* O26 were examined. The properties of these strains are summarized in Table 1. All 37 O26 strains that were of H type 11 (including the VT-positive strains) or non-motile hybridized with the *eae* probe. They also gave localized adhesion to HEp-2 cells accompanied by a positive FAS test. The percentage of cells with clusters of bacteria characteristic of localized adhesion was in the range 0·1–33. In a previous study [10], some VT-positive or VT-negative O26:H11 strains produced E-Hly and hybridized with the CVD419 probe, so all O26 strains were examined for these properties. A proportion of both H11 (14/18) and non-motile (11/19) strains produced E-Hly. They also hybridized with the CVD419 probe, whereas the E-Hly-negative strains did not. Five strains, none of which was VT-positive, produced α-Hly. Two O26:H32 strains were negative in all tests.

Thirty-eight of the O26 strains had been isolated from children aged under 4 years and one from an adult. For only one patient was the diarrhoea known to be associated with blood (and mucus); the 026 strain from this patient was H11, VT-positive and E-Hly-negative. The adult patient had persistent non-bloody diarrhoea of more than 3 weeks duration; the strain from this patient was non-motile and produced E-Hly but not VT. Eight children had diarrhoea known to have lasted longer than 1 week; for five the information was given that this diarrhoea was non-bloody. Strains isolated from four of these patients were of H type 11 and they produced VT1 and E-Hly. Strains from the other four patients were non-motile and VT-negative; three produced E-Hly and one α-Hly.

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Table 1. *Properties of 39* E. coli *O26 strains*

H type no. of strains	Hybridization with probes for				Production of*		Hep-2 cell adhesion	FAS† test
	VT1	VT2	*eae*	CVD 419	E-Hly	α-Hly		
H11								
10	+	−	+	+	+	−	LA	+
3	+	−	+	−	−	−	LA	+
1	−	+	+	+	+	−	LA	+
3	−	−	+	+	+	−	LA	+
1	−	−	+	−	?	+	LA	+
Non-motile								
11	−	−	+	+	+	−	LA	+
4	−	−	+	−	−	−	LA	+
4	−	−	+	−	?	+	LA	+
H32								
2	−	−	−	−	−	−	−	

The strains did not hybridize with the EAF probe.

* E-Hly, enterohaemolysin; α-Hly, α-haemolysin. ?E-Hly could not be demonstrated in the presence of α-Hly.

† The FAS test could not be performed in the absence of adhesion.

Table 2. *Properties of 29* E. coli *O55 strains*

H type	No. of strains	Hybridization with probe for			HEp-2 cell adhesion	FAS* test
		eae	EAF	EAggEC		
H1	1	−	−	−	−	
H7	9	+	−	−	LA	+
	2	+	−	−	−	
H10	1	−	−	+	−	
H24	1	−	−	−	−	
H27	1	−	−	+	Agg	−
H32	2	−	−	−	−	
H34	3	+	−	−	LA	+
H45	1	−	−	−	−	
Non-motile	3	+	−	−	LA	+
	2	+	−	−	−	
	2	+	+	−	LA	+
	1	+	−	−	DA†	−

The strains did not hybridize with the VT1 or VT2 probes.

* The FAS test could not be performed in the absence of adhesion.

† This strain hybridized with the DAEC probe.

Strains of E. coli *O55*

Twenty-nine strains belonging to serogroup O55 were examined. The H types and other properties are shown in Table 2. Strains belonging to H types 7 (11/11), 34 (3/3) or that were non-motile (8/8) hybridized with the *eae* probe. Seventeen of these 22 *eae*-positive strains gave localized adhesion to HEp-2 cells and a

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Table 3. *Properties of 24* E. coli *O111 strains*

H type	No. of strains	Hybridization with probe for *eae*	Hybridization with probe for EAggEC	HEp-2 cell adhesion	FAS* test
H7	4	−	+	Agg	−
H12	1	−	+	Agg	−
	1	−	+	−	
H25	6	+	−	LA	+
	7	+	−	−	
Non-motile	3	−	+	Agg	−
	2	−	−	−	

The strains did not hybridize with the VT1, VT2 or EAF probes.
* The FAS test could not be performed in the absence of adhesion.

positive FAS test. Two of the non-motile strains adhered to 100% of the HEp-2 cells and were the only O55 strains that hybridized with the EAF probe. The remaining 15 strains attached to between 0·5 and 10% of the cells. For four *eae*-positive strains no attachment was seen. One non-motile strain, E73107/1, that hybridized with the *eae* probe gave excellent diffuse adhesion and hybridized with the DA probe; no actin accumulation was seen beneath adhering bacteria in the FAS test. Two O55 strains, that did not hybridize with the *eae* probe, hybridized with the EAggEC probe; of these only E75021 (O55:H27) attached to HEp-2 cells and gave the characteristic aggregative pattern of attachment.

Twenty-six of the O55 strains had been isolated from children aged under 4 years and three from patients aged 7, 9 and 24 years. There were no reports of bloody diarrhoea. Three children had diarrhoea that persisted longer than 1 week. The O55 strains isolated from two of these patients were *eae*-positive, EAF-negative. The aggregative strain, E75021, was isolated from the third child who had suffered diarrhoea up to five times daily for a period of 10 days.

Strains of E. coli *O111*

Twenty-four strains of O111 were examined. The H types and properties of these strains are shown in Table 3. All were of the subgroup O111ab; the strains were VT-negative and we have previously noted that VT-producing O111 strains were of subgroup ac [11]. All 13 strains of H type 25 hybridized with the *eae* but not the EAF probe. Six of these strains adhered in a localized pattern to about 1% of HEp-2 cells and gave a positive FAS test; no attachment was seen for seven strains. Six strains belonging to H types 7 or 12, and three of five non-motile strains hybridized with the EAggEC probe. All but one of these nine strains gave the aggregative pattern of attachment to cells.

Nineteen O111 strains had been isolated from children aged under 4 years and one from an adult aged 29 years. In addition, four strains were from a family outbreak in which the patients were aged 13 months, 6, 8 and 31 years. Entero-aggregative *E. coli* of H type 7 were isolated from all four members of the family. The baby had suffered non-bloody diarrhoea up to four times daily for at least 3 weeks; oocysts of cryptosporidia were also present in the faeces. The other family members were stated to have had diarrhoea but excretion of oocysts was not seen.

Table 4. *Properties of 30* E. coli *O128* strains*

H type	No. of strains	Hybridization with probe for VT1	VT2	*eae*	EAggEC	HEp-2 cell adhesion	FAS test†
H2	17	−	−	+	−	LA	+
	3	+	+	−	−	−	
	1	−	−	−	−	−	
H8	1	−	−	+	−	LA	+
H12	1	−	−	−	−	−	
H35	6	−	−	−	+	−	
Non-motile	1	+	−	−	−	−	

The strains did not hybridize with the EAF probe.

* The O128 strains belonged to subgroup ab, with the exception of one of the 17 *eae*-positive H2 strains and the single strain of H type 12 which were both subgroup ad.

† The FAS test could not be performed in the absence of adhesion.

EAggEC of H type 12 were isolated from the other adult, who was the only patient for which bloody diarrhoea was noted. Four other children were stated to have had diarrhoea for longer than 7 days. The strains isolated from them were O111:H25 *eae*$^+$ LA$^+$, O111:H25 *eae*$^+$ LA$^-$, O111:H− EAgg$^+$ and a non-motile O111 strain that was negative for all properties.

Strains of E. coli *O128*

Thirty strains were examined. The H types and other properties are shown in Table 4. Eighteen strains that were of H types 2 or 8 hybridized with the *eae* probe. These did not include any of the VT-producing strains. The *eae*-positive strains gave localized attachment to between 0·5 and 92% of HEp-2 cells and a positive FAS test but did not hybridize with the EAF probe. Six strains of H type 35 hybridized with the EAggEC probe but showed no attachment to HEp-2 cells.

All 30 O128 strains had been isolated from children aged under 7 years. There were no reports of bloody diarrhoea. Two children were stated to have suffered from persistent diarrhoea. An O128:H35 EAggEC-positive strain was isolated from one child with diarrhoea of 7 weeks duration but for this case there was also evidence of giardia infection. The other case of persistent diarrhoea had lasted for over 2 years and the child exhibited poor weight gain. The O128:H12 strain was isolated from this patient but O128 strains had not been isolated from previous faecal specimens.

DISCUSSION

The initial aim of this study was to obtain information on VTEC belonging to serogroups O26, O55, O111 or O128 from cases of diarrhoea in the United Kingdom in 1991. Of the 122 isolates examined only 14 of 39 O26 strains and 4 of 30 O128 strains were VTEC. We have previously reported the isolation of VT-positive O55 and O111 strains from cases of HUS in the United Kingdom [11] but the results of the present study are similar to that undertaken more than 10 years ago in which, within strains belonging to the classical EPEC serogroups, only strains of O26 and O128 were shown to be VT-positive [9]. Nevertheless the present study did show that the majority of the strains belonging to the four

serogroups under investigation possessed other putative virulence factors. Only 11 of the 104 VT-negative strains examined were negative in all other tests. Seventy-six strains hybridized with the *eae* probe and 17 with the EAggEC probe. Fourteen of the 18 VTEC also hybridized with the *eae*-probe.

VT1, rather than VT2, was produced by all except one of the VT-positive O26:H11 strains in agreement with previous studies [10, 25, 26]. Non-motile O26 strains in this study did not produce VT but resembled O26:H11 strains in possessing the ability to cause AE lesions and to hybridize with the *eae* probe. These properties, and not VT production, appear to be characteristic of the O26 serogroup. Some non-motile O26 strains also resembled VT-positive strains in producing E-Hly and hybridizing with the CVD419 probe. In a survey of VTEC of many different serogroups a similar association between the production of E-Hly and the presence of the plasmid encoding the CVD419 sequence was shown [11]. Other workers have associated E-Hly production with the presence of a bacteriophage [27] and further work on the relationship between plasmid and phage genes is needed. Several O26 strains produced α-Hly and for technical reasons it was not possible to determine if they also produced E-Hly. It was noted that none of these strains hybridized with the CVD419 probe. The association of E-Hly production and hybridization with the CVD419 probe contrasts with the lack of association between VT-production and hybridization with the CVD419 probe that we have noted in this and other studies [10, 11].

Only two non-motile O55 strains hybridized with the EAF probe (in addition to the *eae* probe) and so resembled EPEC strains isolated from outbreaks [22, 28, 29]. The ability of some strains belonging to classic EPEC serogroups to cause characteristic AE lesions in the intestine has been shown for humans and in animal models [30–32]. Both plasmid and chromosomal genes are necessary for full virulence and these include the *eae* and EAF genes. An earlier study [33] showed that EAF-positive strains were uncommon amongst EPEC isolated from sporadic cases of diarrhoea in the United Kingdom during 1985–7. We have since shown that many of these EAF-negative strains gave LA and a positive FAS test [10, 34, 35] and that they hybridize with the *eae* probe (unpublished results). Similarly for all four serogroups (O26, O55, O111 and O128) in the present study, with only two exceptions, the *eae*-positive strains were EAF-negative. For most of the 88 *eae*-positive EAF-negative strains it was confirmed that they gave LA and a positive FAS test but 11 strains gave no attachment and so a FAS test result was not possible. The *eae*-positive EAF-negative strains may not be a homogeneous group as some give consistently good LA in tissue culture tests whilst others do not. For some [34] it is known that they carry plasmid encoded genes that are important for attachment and in this resemble EAF-positive strains; for most, this information is not available. Knutton and coworkers [36] observed that FAS-positive EAF-negative strains that attached poorly to HEp-2 cells nevertheless adhered well to human small intestinal mucosa. Volunteer studies, similar to those performed for EAF-positive organisms [37], are needed to confirm that the various groups of *eae*-positive EAF-negative *E. coli* are diarrhoeagenic and to compare the severity of any infections.

Nine strains belonging to serogroups O55 and O111 were identified as belonging to the aggregative class of *E. coli*; they gave characteristic adhesion to HEp-2 cells

and hybridized with the EAggEC probe. We earlier reported that strains belonging to EPEC serotypes O44:H18, O111:H21 and O126:H27 and isolated in the United Kingdom may also be EAggEC [35]. Epidemiological studies in other countries have shown a statistically significant association of EAggEC with cases of persistent diarrhoea [38, 39]. Similar studies are needed in the UK but several patients with persistent diarrhoea in this study did excrete these organisms. Eight strains, including all six O128:H35 strains, also hybridized with the EAggEC probe but no adhesion to HEp-2 cells could be demonstrated. This difference between the two tests is being investigated further.

In this study only one strain, E73107/1 (serotype O55:H−), was identified as diffusely adherent. Unlike most DAEC (unpublished observations) E73107/1 was unusual in hybridizing with the *eae* probe. Cantey and Moseley [40] observed that the introduction of genes encoding DA into poorly adhering *eae*-positive *E. coli* strains resulted in good DA accompanied by a positive FAS test. Although the actin aggregation was considered to be more widely distributed than that seen beneath an EPEC control, the results supported the hypothesis that an adhesin such as that resulting in DA, and not only that associated with the EAF plasmid, could bring the bacterium into close proximity to the cell and allow the expression of the *eae* gene. The negative FAS test with E73107/1 contrasts with these results and it is possible that this strain lacks other genes required for actin accumulation.

Historically EPEC were defined as strains belonging to certain serogroups that, generally, had been associated with outbreaks of infantile gastroenteritis. However since 1970 such outbreaks have virtually disappeared in developed countries. Nevertheless use of the EPEC antisera has continued for the investigation of sporadic cases of diarrhoea and recently their validity to detect pathogenic strains has been questioned [41]. Our results show that strains selected by the use of O26, O55, O111 and O128 antisera contain a high proportion of strains (111 of 122) with putative virulence factors, some of which have only recently been described. There is a need to obtain similar information on strains belonging to the other classical EPEC serogroups. Finally it is necessary to confirm whether the strains possessing the putative virulence factors can cause diarrhoea and this might be done by challenge experiments or epidemiological studies.

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REFERENCES

1. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; **2**: 15–38.
2. Smith HR, Scotland SM. Vero cytotoxin-producing strains of *Escherichia coli*. J Med Microbiol 1988; **26**: 77–85.
3. Riley LW. The epidemiologic, clinical, and microbiologic features of hemorrhagic colitis. Ann Rev Microbiol 1987; **41**: 383–407.
4. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–98.

5. Bitzan M, Karch H, Maas MG, et al. Clinical and genetic aspects of shiga-like toxin production in traditional enteropathogenic *Escherichia coli*. Zentralbl Bakteriol 1991; **274**: 496–506.
6. Bopp CA, Greene KD, Downes FP, Sowers EG, Wells JG, Wachsmuth IK. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. J Clin Microbiol 1987; **25**: 1486–9.
7. Katouli M, Kühn I, Möllby R. Biochemical phenotypes of enteropathogenic *Escherichia coli* common to Iran and Sweden. J Med Microbiol 1991; **35**: 270–7.
8. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977; **18**: 775–9.
9. 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**: 15–7.
10. Scotland SM, Willshaw GA, Smith HR, Rowe B. Properties of strains of *Escherichia coli* O26:H11 in relation to their enteropathogenic or enterohemorrhagic classification. J Infect Dis 1990; **162**: 1069–74.
11. Willshaw GA, Scotland SM, Smith HR, Rowe B. Properties of Vero cytotoxin-producing *Escherichia coli* of human origin belonging to O-serogroups other than O157. J Infect Dis 1992; **166**: 797–802.
12. 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.
13. 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.
14. Thomas A, Smith HR, Willshaw GA, Rowe B. Non-radioactively labelled polynucleotide and oligonucleotide DNA probes, for selectively detecting *Escherichia coli* strains producing Vero cytotoxins VT1, VT2 and VT2 variant. Molec Cell Probes 1991; **5**: 129–35.
15. 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.
16. Baldini MM, Nataro JP, Kaper JB. Localization of a determinant for HEp-2 adherence by enteropathogenic *Escherichia coli*. Infect Immun 1986; **52**: 334–6.
17. Jerse AE, Yu J, Tall BD, Kaper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci USA 1990; **87**: 7839–43.
18. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J Infect Dis 1990; **161**: 1249–51.
19. Bilge SS, Clausen CR, Lau W, Moseley SL. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J Bacteriol 1989; **171**: 4281–9.
20. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
21. Moseley SL, Hardy JW, Huq MI, Echeverria P, Falkow S. Isolation and nucleotide sequence determination of a gene encoding a heat-stable enterotoxin of *Escherichia coli*. Infect Immun 1983; **39**: 1167–74.
22. Scotland SM, Willshaw GA, Smith HR, Gross RJ, Rowe B. Adhesion to cells in culture and plasmid profiles of enteropathogenic *Escherichia coli* isolated from outbreaks and sporadic cases of infant diarrhoea. J Infect 1989; **19**: 237–49.
23. Knutton S, Baldwin T, Williams PH, McNeish AS. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect Immun 1989; **57**: 1290–8.
24. Willshaw GA, Smith HR, McConnell MM, et al. Plasmid-encoded production of coli-surface associated antigen 1 (CS1) in a strain of *Escherichia coli* serotype O139.H28. Microb Path 1990: **9**: 1–11.
25. Beutin L, Montenegro MA, Ørskov I, et al. Close association of verotoxin (Shiga-like toxin) production with enterohaemolysin production in strains of *Escherichia coli*. J Clin Microbiol 1989; **27**: 2559–64.

26. Levine MM, Prado V, Kaper JB, et al. DNA probes to detect diarrheagenic *Escherichia coli*. In: Balows A, Tilton RC, Turano A, eds. Rapid methods and automation in microbiology and immunology. Brescia, Italy: Brixia Academic Press, 1989: 420–7.
27. Beutin L, Bode L, Ozel M, Stephan R. Enterohemolysin production is associated with a temperate bacteriophage in *Escherichia coli* serogroup O26 strains. J Bacteriol 1990; **172**: 6469–75.
28. Jerse AE, Kaper JB. The *eae* gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. Infect Immun 1991; **59**: 4302–9.
29. Moyenuddin M, Wachsmuth IK, Moseley SL, Bopp CA, Blake PA. Serotype, antimicrobial resistance, and adherence properties of *Escherichia coli* strains associated with outbreaks of diarrheal illness in children in the United States. J Clin Microbiol 1989; **27**: 2234–9.
30. Knutton, S, Lloyd DR, McNeish AS. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect Immun 1987: **55**: 69–77.
31. Rothbaum R, McAdams AJ, Giannella R, Partin JC. A clinicopathologic study of enterocyte-adherent *Escherichia coli*: a cause of protracted diarrhea in infants. Gastroenterology 1982; **83**: 441–54.
32. Tzipori S, Gibson R, Montanaro J. Nature and distribution of mucosal lesions associated with enteropathogenic and enterohemorrhagic *Escherichia coli* in piglets and the role of plasmid-mediated factors. Infect Immun 1989; **57**: 1142–50.
33. Smith HR, Scotland SM, Stokes N, Rowe B. Examination of strains belonging to enteropathogenic *Escherichia coli* serogroups for genes encoding EPEC adherence factor and Vero cytotoxins. J Med Microbiol 1990; **31**: 235–40.
34. Scotland SM, Smith HR, Rowe B. *Escherichia coli* O128 strains from infants with diarrhea commonly show localized adhesion and positivity in the fluorescent-actin staining test but do not hybridize with an enteropathogenic *E. coli* adherence factor probe. Infect Immun 1991; **59**: 1569–71.
35. Scotland SM, Smith HR, Said B, Willshaw GA, Cheasty T, Rowe B. Identification of enteropathogenic *Escherichia coli* isolated in Britain as enteroaggregative or as members of a subclass of attaching-and-effacing *E. coli* not hybridising with the EPEC adherence-factor probe. J Med Microbiol 1991; **35**: 278–83.
36. Knutton S, Phillips AD, Smith HR, et al. Screening for enteropathogenic *Escherichia coli* in infants with diarrhea by the fluorescent-actin staining test. Infect Immun 1991; **59**: 365–71.
37. Levine MM, Bergquist EJ, Nalin DR, et al. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet 1978: **i**: 1119–22.
38. Bhan MK, Raj P, Levine MM, et al. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J Infect Dis 1989; **159**: 1061–4.
39. Cravioto A, Tello A, Navarro A, et al. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhoea. Lancet 1991; **337**: 262–4.
40. Cantey JR, Moseley SL. HeLa cell adherence, actin aggregation, and invasion by nonenteropathogenic *Escherichia coli* possessing the *eae* gene. Infect Immun 1991; **59**: 3924–9.
41. Morris KJ, Rao GG. Conventional screening for enteropathogenic *Escherichia coli* in the UK. Is it appropriate or necessary? J Hosp Infect 1992; **21**: 163–7.