*Journal of Medical Microbiology* (2004), **53**, 1145–1149 **DOI** 10.1099/jmm.0.45684-0

# Distribution of *espI* among clinical enterohaemorrhagic and enteropathogenic *Escherichia coli* isolates

Rosanna Mundy,[1] Claire Jenkins,[2] Jun Yu,[1] Henry Smith[3] and Gad Frankel[1]

Correspondence
Gad Frankel
g.frankel@imperial.ac.uk

[1]Centre for Molecular Microbiology and Infection, Department of Biological Sciences, Imperial College London, London SW7 2AZ, UK

[2]Microbiology Department, Royal Free Hospital, Pond Street, London NW3 2QG, UK

[3]Laboratory of Enteric Pathogens, Specialist and Reference Microbiology Division, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK

Received 25 March 2004
Accepted 22 July 2004

Enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* are important diarrhoeagenic pathogens; infection is dependent on translocation of a number of type III effector proteins. Until recently all the known effectors were encoded on the LEE pathogenicity island, which also encodes the adhesin intimin and the type III secretion apparatus. Recently, a novel non-LEE effector protein, EspI/NleA, which is required for full virulence *in vivo* and is encoded on a prophage, was identified. The aim of this study was to determine the distribution of *espI* among clinical EHEC and EPEC isolates. *espI* was detected in 86 % and 53 % of LEE$^+$ EHEC and EPEC strains, respectively. Moreover, the *espI* gene was more commonly found in patients suffering from a more severe disease.

## INTRODUCTION

Enteric bacteria have a conserved core genomic structure, common to both commensal and pathogenic strains, that provides micro-organisms with mechanisms required for survival in the competitive gut, as well as the ability to transmit between hosts and survive in the environment (Dougan *et al.*, 2001). In pathogenic bacteria, the core genome framework is further decorated with novel genetic islands often associated with enhanced virulence (Wain *et al.*, 2001). Striking examples of such pathogenic bacteria are the diarrhoeal agents enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC), also known as Verocytotoxin-producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) (Nataro & Kaper, 1998).

EPEC is a frequent cause of infantile diarrhoea in the developing world while EHEC causes a wide spectrum of illnesses ranging from mild diarrhoea to severe diseases, such as haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Strains of EHEC belonging to serogroup O157 are most commonly associated with severe human disease (Willshaw *et al.*, 2001). However, infections with EHEC of other serogroups (i.e. O26, O103, O111 and O145) have also been documented (Caprioli *et al.*, 1997).

Abbreviations: EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; HUS, haemolytic uraemic syndrome; IID, Infectious Intestinal Disease; LEP, Laboratory of Enteric Pathogens.

By adhering to intestinal epithelial cells, EHEC and EPEC produce a histopathological feature known as the attaching and effacing (A/E) lesion (reviewed by Frankel *et al.*, 1998), which is characterized by localized destruction of brush border microvilli and intimate attachment of the bacteria to the plasma membrane of the host epithelial cells. The capacity to form A/E lesions is encoded on the LEE pathogenicity island (McDaniel *et al.*, 1995), which encodes the positive regulator Ler (Mellies *et al.*, 1999), the outer-membrane adhesin intimin (Jerse *et al.*, 1990), a type III secretion system (TTSS) and effector proteins (Tir, EspF, Map, EspG, EspH) (Elliott *et al.*, 2001; Kenny *et al.*, 1997; Kenny & Jepson, 2000; McNamara & Donnenberg, 1998; Tu *et al.*, 2003) that are translocated into the epithelial cell for the benefit of the pathogen.

Recently, we and others identified a novel type III secreted protein, termed EspI (Mundy *et al.*, 2004) or NleA (Gruenheid *et al.*, 2004), that was found in the genome sequence of EHEC (Hayashi *et al.*, 2001; Perna *et al.*, 2001) and EPEC (http://www.sanger.ac.uk/projects/Escherichia_Shigella) to be encoded on a prophage outside of the LEE region. An *espI*/*nleA* homologue is found on Stx1-converting phage phi-4795 in EHEC strain O84 (AJ487680) but is missing from laboratory *E. coli* strains (Gruenheid *et al.*, 2004). Importantly, despite not affecting A/E lesion formation *in vitro* (Gruenheid *et al.*, 2004; Mundy *et al.*, 2004), EspI was shown to be essential for virulence *in vivo* using the

*Citrobacter rodentium* mouse model of infection (Mundy *et al.*, 2004; Gruenheid *et al.*, 2004), which became a popular surrogate model for *in vivo* studies of the mechanisms and processes of A/E *E. coli* pathogenesis. The aim of this study was to determine the prevalence and distribution of *espI* among clinical EPEC and EHEC isolates.

## METHODS

**Bacterial strains.** Ninety-three strains of EHEC, 29 from patients with HUS, 49 from patients with diarrhoea who did not develop HUS and 15 from a healthy control group, were obtained from the culture collection of the Laboratory of Enteric Pathogens (LEP), London. Strains from patients with HUS and diarrhoea were isolated between 1968 and 2000 either at the LEP from faecal samples sent in for tests for EHEC and EPEC, or at hospital laboratories in the UK, and sent to the LEP for confirmation and further tests (Jenkins *et al.*, 2003a, b; Kleanthous *et al.*, 1990; Willshaw *et al.*, 1992, 2001). The strains from the healthy control group were isolated during a study on Infectious Intestinal Disease (IID) in England (Evans *et al.*, 2002). Healthy controls were defined as individuals without loose stools or significant vomiting for 3 weeks before the faecal sample was taken. Twenty-five strains of EPEC were from patients with diarrhoea sent to the LEP for identification and further characterization between January and December 2000, and a further 207 strains were isolated during the IID study (Tompkins *et al.*, 1999) from cases and control. The strains isolated during the IID study were from cases and controls in two study components: one was a community cohort and the other was a General Practice case-control component. Cases were individuals with loose stools and significant vomiting lasting less than 2 weeks, in the absence of a known non-infectious cause and proceeded by a symptom-free period of 3 weeks. Controls were individuals without loose stools or significant vomiting for 3 weeks.

**Table 1.** Strains of EPEC associated with cases of sporadic diarrhoeal disease between January and December 2000

EPEC strains were defined as being LEE$^+$ and *vtx*$^-$.

| Serotype* | *eae* type† | *espI* |
|---|---|---|
| O26 : H11 (1) | $\beta$ | + |
| O26 : H− (1) | $\beta$ | + |
| O55 : H34 (1) | $\gamma$ | + |
| O101 : H− (1) | $\gamma$ | + |
| O116 : H33 (1) | $\gamma$ | − |
| O116 : H33 (1) | $\gamma$ | + |
| O119 : H2 (1) | $\beta$ | + |
| O128ab : H2 (1) | $\beta$ | + |
| O128ab : H2 (1) | $\beta$ | − |
| O156 : H8 (1) | $\theta$ | − |
| O? : H10 (1) | $\beta$ | + |
| O? : H11 (1) | U | − |
| O? : H11 (1) | $\beta$ | + |
| O? : H25 (1) | $\zeta$ | + |
| O? : H27 (1) | U | − |
| O? : H33 (1) | $\gamma$ | + |
| O? : H33 (1) | $\gamma$ | + |
| O? : H33 (1) | U | − |
| O? : H− (3) | $\varepsilon$ | + |
| O? : H− (2) | U | − |
| O? : H− (1) | $\zeta$ | − |
| O rough : H34 (1) | $\zeta$ | − |

*Number of strains isolated in parentheses. O?, Strains that could not be serogrouped using the LEP serotyping scheme; O rough, strains that do not express the 'O' antigen and therefore cannot be typed using a phenotypic serotyping scheme.

†U, Strains could not be typed as the subtyping PCR failed to amplify sufficient DNA for the test.

**DNA hybridization.** The *espI* gene was amplified by PCR using Hotstar Taq (Invitrogen) from EHEC O157 : H7 (EDL933) using the forward primer 5′-ATGAACATTCAACCGACCATACAATCTG and the reverse primer 5′-TTAGACTCTTGTTTCTTGGATTATATCA. The amplified 1293 bp DNA fragment was purified using a PCR Clean-up kit (Qiagen) and labelled with fluorescein-deoxyuridine triphosphate (dUTP) using a random primer labelling kit (Amersham) according to the manufacturer's instructions. The *eae* gene and the EAF plasmid were detected using DNA probes described by Jerse *et al.* (1990) and Nataro *et al.* (1985). These probes were also labelled with fluorescein dUTP.

Probe tests were performed according to the method of Maniatis *et al.* (1982). Briefly, broth cultures were spotted on nylon membranes. The membranes were overlaid on nutrient agar, incubated for 6 h and, after alkaline lysis, the DNA was bound to the membrane. Filters were hybridized overnight at 68 °C with the fluorescein-labelled probes. Stringency washes were carried out at 68 °C. Colonies harbouring the target genes were detected using enhanced chemiluminescence, as described by the manufacturer (Amersham).

**Subtyping of the *eae* gene.** The subtyping of the *eae* gene was performed using the methods described previously (Jenkins *et al.*, 2003a). The *eae* gene subtypes of some of the strains have been reported (Jenkins *et al.*, 2003a, c).

**Statistics.** The chi-square ($\chi^2$) test with the Yates' correction was used to determine if there were any statistically significant differences in the distribution of the *espI* gene between groups of EPEC and EHEC strains. A $\chi^2$ test of association was carried out to assess the relationship between the presence of the *espI* gene and the presence of the *eae* gene. $\chi^2$ tests were also performed to determine the relationship between the *espI* gene and disease symptoms in patients with EHEC. In all cases, a $P$ value of $< 0{\cdot}05$ was taken to indicate significance.

**Table 2.** Association of *espI* with intimin subtypes in 207 strains of EPEC from the IID study

| *espI* gene +ve | *eae* type | *espI* gene −ve |
|---|---|---|
| 3 | $\alpha$ | 30 |
| 30 | $\beta$ | 9 |
| 11 | $\gamma$ | 21 |
| 29 | $\gamma 2$ | 23 |
| 17 | $\zeta$ | 10 |
| 4 | $\varepsilon$ | 2 |
| 15 | $\delta$ | 3 |
| Total 109 | | 98 |

**Table 3.** Presence of *espI* in strains of EHEC

| Serotype* | Symptom† | *vtx* type‡ | *eae* type | *espI* |
|---|---|---|---|---|
| O4 : H10 (1) | D | 1+2 | – | – |
| O5 : H– (2) | HUS (1), D (1) | 1 | *β* | + |
| O9ab : H– (1) | HUS | 2 | – | – |
| O26 : H11 (8) | HUS (3), D (5) | 1 | *β* | + |
| O26 : H11 (1) | D | 1 | *β* | – |
| O52 : H52 (1) | D | 2 | – | – |
| O55 : H7 (1) | HUS | 2 | *γ* | + |
| O55 : H10 (1) | HUS | 2 | – | – |
| O76 : H7 (1) | D | 2 | *γ* | + |
| O82 : H2 (1) | A | 1+2 | – | – |
| O91 : H– (2) | D (1), A (1) | 1+2 (1), 1 (1) | – | – |
| O101 : H– (1) | HUS | 2 | – | – |
| O104 : H2 (1) | HUS | 2 | – | – |
| O105ac : H18 (2) | HUS (1), D (1) | 1+2 | – | – |
| O111ac : H– (1) | HUS | 1+2 | *θ* | + |
| O115 : H10 (2) | HUS (1), A (1) | 1 | – | – |
| O118 : H12 (4) | D (3), A (1) | 2 | – | – |
| O128ab : H– (2) | D | 2 | *β* | – |
| O128ab : H– (1) | D | 1+2 | – | – |
| O128ab : H2 (10) | HUS (1), D (6), A (3) | 1+2 | – | – |
| O128ab : H7 (1) | HUS | 2 | *γ* | – |
| O128ab : H8 (1) | D | 1 | – | – |
| O128ab : H25 (1) | HUS | 2 | *β* | + |
| O134 : H25 (1) | HUS | 2 | – | – |
| O145 : H– (1) | HUS | 1 | *γ* | + |
| O145 : H25 (4) | HUS | 2 | *β* | + |
| O146 : H21 (2) | A | 1+2 | – | – |
| O157 : H– (4)§ | D | 2 | *γ* | + |
| O157 : H7 (12) | D | 2 (6), 1+2 (6) | *γ* | + |
| O162 : H6 (1) | A | 1+2 | – | – |
| O162 : H8 (1) | A | 1+2 | – | – |
| O163 : H19 (2) | HUS | 2 | – | – |
| O165 : H25 (1) | HUS | 2 | *ε* | + |
| O168 : H– (1) | HUS | 2 | – | – |
| O173 : H21 (1) | HUS | 2 | – | – |
| E55992/88 : H– (1)‖ | HUS | 1+2 | – | – |
| O? : H– (1) | D | 1 | *β* | + |
| O? : H– (2) | A | 2 (1), 1+2 (1) | – | – |
| O? : H2 (1) | D | 1 | *ε* | – |
| O? : H10 (2) | D | 2 (1), 1+2(1) | – | – |
| O? : H18 (1) | A | 1+2 | – | – |
| O? : H19 (1) | D | 1 | – | – |
| O? : H21 (2) | HUS (1), A (1) | 2 (1), 1 (1) | – | – |
| O? : H40 (1) | HUS | 2 | *θ* | – |
| O rough : H– (1) | D | 2 | – | – |
| O rough : H– (1) | D | 1+2 | – | – |
| O rough : H45 (1) | D | 2 | – | – |

*Number of strains isolated in parentheses. O?, Strains that could not be serogrouped using the LEP serotyping scheme; O rough, strains that do not express the 'O' antigen and therefore cannot be typed using a phenotypic serotyping scheme.

†D, Diarrhoea; A, asymptomatic.

‡*vtx*, Verocytotoxin gene.

§Sorbitol-fermenting strain.

‖Provisional new serotype.

## RESULTS AND DISCUSSION

We assembled a large collection of strains, isolated from patients with HUS and diarrhoeal disease and from a healthy control group, and tested the strains for the presence of *espI* using a specific DNA probe. Two hundred and thirty-two EPEC strains were tested for the presence of *espI* using colony hybridizations. *espI* was detected in 124 of the 232 (53·4 %) strains. Table 1 shows the distribution of *espI* in the 25 EPEC strains from the LEP culture collection. Nine of the 232 EPEC strains carried the EPEC adherence factor (EAF) plasmid and all of these isolates also had the *espI* gene. There was no difference in the prevalence of *espI* between strains isolated from either cases or controls in the IID study and there was no association between *espI* and defined EPEC serogroups. However, the *espI* gene was more commonly associated with strains harbouring the $\beta$-, $\delta$-, $\gamma$2- or $\zeta$-intimin genes than with strains harbouring the $\alpha$- or $\gamma$-intimin genes (Table 2).

Ninety-three strains of EHEC were tested for the presence of the *espI* gene, 43 of which had the *eae* gene (LEE$^+$). *espI* was detected in 37 of the 43 (86 %) EHEC intimin-positive isolates, which included 16 EHEC O157 and 8 EHEC O26 strains, and in none of the intimin-negative (LEE$^-$) strains (Table 3). Therefore, the *espI* gene appears to be highly associated with the presence of the *eae* gene in EHEC ($\chi^2 = 67{\cdot}9$, with Yates' correction; $df = 1$, $P < 0{\cdot}0001$). However, there was no correlation between the presence of *espI* and a specific intimin type. Sixteen of 19 (84·2 %) intimin $\beta$ strains were positive for *espI* and 19 of 20 (95·0 %) intimin $\gamma$ strains were positive for *espI* [$\chi^2 = 0{\cdot}34$, with Yates' correction ($df = 1$, $P = 0{\cdot}561$), showing there is no statistically significant association]. As only two strains with the $\theta$-intimin and two strains with the $\varepsilon$-intimin gene were isolated, no statistical analysis could be carried out to look for an association between these intimin subtypes and the presence of the *espI* gene.

The *espI* gene was also more commonly found in patients with more severe disease. Thirteen of 29 (44·8 %) strains isolated from patients with HUS and 24 of 49 (49 %) strains isolated from patients with diarrhoea contained *espI*, compared to none of the 15 strains isolated from asymptomatic carriers. Statistical analysis confirmed that *espI* is more frequently associated with strains isolated from symptomatic than from asymptomatic individuals, both from those with severe disease (HUS) ($\chi^2 = 7{\cdot}52$, with Yates' correction; $df = 1$, $P < 0{\cdot}006$) and from those with diarrhoea ($\chi^2 = 9{\cdot}758$, with Yates' correction; $df = 1$, $P < 0{\cdot}002$).

EspI is a member of a growing family of newly discovered type III secreted proteins of A/E *E. coli* (AEEC) that are not encoded on the LEE region (Marches *et al.*, 2003). EspI is a type III secreted effector protein that is essential for full virulence *in vivo* (Mundy *et al.*, 2004; Gruenheid *et al.*, 2004) and is found more frequently among strains isolated from humans suffering from a symptomatic EHEC infection. The fact that *espI* is found more frequently in LEE$^+$ EHEC (86 %) than EPEC (53·4 %) strains suggests that *espI* might also play a role during bacterial spread in the environment or in the animal reservoir. We intend to test this hypothesis experimentally by engineering an *espI* EHEC mutant that will be tested in a calf model of EHEC infection (Stevens *et al.*, 2002).

## ACKNOWLEDGEMENTS

This project was supported by the BBSRC and the Wellcome Trust (as part of the International Partnership Research Award in Veterinary Epidemiology project entitled *Epidemiology and Evolution of Enterobacteriaceae Infections in Humans and Domestic Animals*).

## REFERENCES

**Caprioli, A., Tozzi, A. E., Rizzoni, G. & Karch, H. (1997).** Non-O157 Shiga toxin-producing *Escherichia coli* infections in Europe. *Emerg Infect Dis* **3**, 578–579.

**Dougan, G., Haque, A., Pickard, D., Frankel, G., O'Goara, P. & Wain, J. (2001).** The *Escherichia coli* gene pool. *Curr Opin Microbiol* **4**, 90–94.

**Elliott, S. J., Krejany, E. O., Mellies, J. L., Robins-Browne, R. M., Sasakawa, C. & Kaper, J. B. (2001).** EspG, a novel type III system-secreted protein from enteropathogenic *Escherichia coli* with similarities to VirA of *Shigella flexneri*. *Infect Immun* **69**, 4027–4033.

**Evans, J., Wilson, A., Willshaw, G. A., Cheasty, T., Tompkins, D. S., Wheeler, J. G. & Smith, H. R. (2002).** Vero cytotoxin-producing *Escherichia coli* in a study of infectious intestinal disease in England. *Clin Microbiol Infect Dis* **8**, 183–186.

**Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B. & Knutton, S. (1998).** Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Mol Microbiol* **30**, 911–921.

**Gruenheid, S., Sekirov, I., Thomas, N. A. & 10 other authors (2004).** Identification and characterization of NleA, a non-LEE encoded type III translocated virulence factor of enterohaemorrhagic *Escherichia coli* O157 : H7. *Mol Microbiol* **51**, 1233–1249.

**Hayashi, T., Makino, K., Ohnishi, M. & 19 other authors (2001).** Complete genome sequence of enterohemorrhagic *Escherichia coli* O157 : H7 and genomic comparison with a laboratory strain K-12. *DNA Res* **28**, 11–22.

**Jenkins, C., Lawson, A. J., Cheasty, T., Willshaw, G. A., Wright, P., Dougan, G., Frankel, G. & Smith, H. R. (2003a).** Subtyping intimin genes from enteropathogenic *Escherichia coli* associated with outbreaks and sporadic cases in the United Kingdom and Eire. *Mol Cell Probes* **17**, 149–156.

**Jenkins, C., Perry, N., Cheasty, T. & 7 other authors (2003b).** Distribution of the *saa* gene in strains of Shiga toxin-producing *Escherichia coli* of human and bovine origin. *J Clin Microbiol* **41**, 1775–1778.

**Jenkins, C., Willshaw, G. A., Evans, J., Cheasty, T., Chart, H., Shaw, D. J., Dougan, G., Frankel, G. & Smith, H. R. (2003c).** Subtyping of virulence genes in verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. *J Med Microbiol* **52**, 941–947.

**Jerse, A. E., Yu, J., Tall, B. D. & Kaper, J. B. (1990).** A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci U S A* **87**, 7839–7843.

**Kenny, B. & Jepson, M. (2000).** Targeting of an enteropathogenic *Escherichia coli* (EPEC) effector protein to host mitochondria. *Cell Microbiol* **2**, 579–590.

**Kenny, B., DeVinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. & Finlay, B. B. (1997).** Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* **91**, 511–520.

**Kleanthous, H., Smith, H. R., Scotland, S. M., Gross, R. J., Rowe, B.,**

**Taylor, C. M. & Milford, D. V. (1990).** Haemolytic uraemic syndromes in the British Isles, 1985-1988: association with verocytotoxin producing *Escherichia coli*. Part 2: microbiological aspects. *Arch Dis Child* **65**, 722–727.

**Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982).** *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

**Marches, O., Ledger, T. N., Boury, M. & 8 other authors (2003).** Enteropathogenic and enterohaemorrhagic *Escherichia coli* deliver a novel effector called Cif, which blocks cell cycle G2/M transition. *Mol Microbiol* **50**, 1553–1567.

**McDaniel, T. K., Jarvis, K. G., Donnenberg, M. S. & Kaper, J. B. (1995).** A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci U S A* **92**, 1664–1668.

**McNamara, B. P. & Donnenberg, M. S. (1998).** A novel proline-rich protein, EspF, is secreted from enteropathogenic *Escherichia coli* via the type III export pathway. *FEMS Microbiol Lett* **166**, 71–78.

**Mellies, J. L., Elliott, S. J., Sperandio, V., Donnenberg, M. S. & Kaper, J. B. (1999).** The Per regulon of enteropathogenic *Escherichia coli*: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). *Mol Microbiol* **33**, 296–306.

**Mundy, M., Petrovska, L., Smollett, K. & 8 other authors (2004).** Identification of a novel *Citrobacter rodentium* type III secreted protein, EspI, and the roles of this and other secreted proteins in infection. *Infect Immun* **72**, 2288–2302.

**Nataro, J. P. & Kaper, J. B. (1998).** Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* **11**, 142–201.

**Nataro, J. P., Baldini, M. M., Kaper, J. B., Black, R. E., Bravo, N. & Levine, M. M. (1985).** Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. *J Infect Dis* **152**, 560–565.

**Perna, N. T., Plunkett, G., 3rd, Burland, V. & 25 other authors (2001).** Genome sequence of enterohaemorrhagic *Escherichia coli* O157 : H7. *Nature* **409**, 529–533.

**Stevens, M. P., van Diemen, P. M., Frankel, G., Phillips, A. D. & Wallis, T. S. (2002).** Efa1 influences colonization of the bovine intestine by shiga toxin-producing *Escherichia coli* serotypes O5 and O111. *Infect Immun* **70**, 5158–5166.

**Tompkins, D. S., Hudson, M. J., Smith, H. R. & 8 other authors (1999).** A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Commun Dis Public Health* **2**, 108–113.

**Tu, X., Nisan, I., Yona, C., Hanski, E. & Rosenshine, I. (2003).** EspH, a new cytoskeleton-modulating effector of enterohaemorrhagic and enteropathogenic *Escherichia coli*. *Mol Microbiol* **47**, 595–606.

**Wain, J., House, D., Pickard, D., Dougan, G. & Frankel, G. (2001).** Acquisition of virulence-associated factors by the enteric pathogens *Escherichia coli* and *Salmonella enterica*. *Philos Trans R Soc Lond B Biol Sci* **356**, 1027–1034.

**Willshaw, G. A., Scotland, S. M., Smith, H. R. & Rowe, B. (1992).** Properties of Verocytotoxin-producing *Escherichia coli* of human origin of O-serogroups other than O157. *J Infect Dis* **166**, 797–802.

**Willshaw, G. A., Cheasty, T., Smith, H. R., O'Brien, S. J. & Adak, G. K. (2001).** Verocytotoxin-producing *Escherichia coli* (VTEC) O157 and other VTEC from human infections in England and Wales: 1995 to 1998. *J Med Microbiol* **50**, 135–142.