Diarrhoeagenic *Escherichia coli* from routine diagnostic faecal samples in England and Wales

There are five established groups of *Escherichia coli* known to be associated with infectious intestinal disease (IID): verocytotoxin-producing *E. coli* (VTEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAggEC) (Kaper *et al.*, 2004). In the UK, VTEC O157 are identified by their inability to ferment sorbitol. However, phenotypically non-O157 VTEC and the other diarrhoeagenic *E. coli* (DEC) are more difficult to distinguish from non-pathogenic *E. coli*, and are not detected routinely.

Specific PCRs designed to detect certain pathogenicity genes can be used to identify strains belonging to the five DEC groups. The pathogenicity genes targeted include the verocytotoxin gene (*vtx*) for the detection of VTEC, the *E. coli* attaching and effacing gene (*eae*) associated with EPEC, the heat-stable (ST) and heat-labile toxin genes (LT) for the identification of ETEC, the invasins plasmid antigen gene (*ipaH*) for EIEC, and the antiaggregation protein transporter gene (*aat*) for the detection of EAggEC (Kaper *et al.*, 2004).

In 1999, a study of IID in England found that of the five DEC groups, EAggEC were isolated most frequently. Strains of EAggEC were identified in 5% of faecal samples with a higher incidence of colonization in cases compared to healthy individuals (Tompkins *et al.*, 1999). Subsequent studies supported these data (Amar *et al.*, 2004; Jenkins *et al.*, 2006). The authors of the IID study recommended 'a review of organisms routinely sought in diagnostic laboratories, and that particular consideration should be given to enteroaggregative ... *E. coli*' (FSA, 2000). Strains of EAggEC are characterized phenotypically by their adherence to HEp-2 cells in a stacked brick formation (Nataro *et al.*, 1987). This phenotype is linked to, but not exclusively

associated with, the presence of the *aat* gene (Baudry *et al.*, 1990).

The Gastrointestinal Infections Reference Unit (GIRU) at the Health Protection Agency receives approximately 1600 isolates each year for confirmation of identification of *Shigella* species, approximately 6% of these are identified as *E. coli*. A further 1600 isolates are submitted per annum as VTEC O157, of which approximately 10% are found to be *vtx*-negative *E. coli*. The aim of this study was to use PCR to determine whether these strains of *E. coli* harboured the *ipaH* and *aat* genes associated with EIEC and EAggEC, respectively.

Between April and September 2009, 85 isolates of *vtx*-negative *E. coli* were identified by GIRU. A total of 44 of these strains were received requesting confirmation or exclusion of VTEC O157 and 41 were submitted requesting confirmation or exclusion of *Shigella* species. All 85 strains were tested for the presence of *ipaH* (Sethabutr *et al.*, 1993) and *aat* (Schmidt *et al.*, 1995; Amar *et al.*, 2005). Of these, 5 were EIEC and 15 were EAggEC (Table 1).

Seven of the EAggEC strains belonged to serogroup O151, two were O62, one was O55 and five were O-unidentifiable. A search of the GIRU archives showed that over the past 5 years a further 18 strains of *E. coli* O151 (9) and O62 (9) had been identified. Fourteen of these harboured the *aat* gene (Table 1). Of the 34 patients from whom EIEC or EAggEC were detected (Table 1), 20 were known to have travelled abroad. The IID study previously showed an association with EAggEC and foreign travel (FSA, 2000).

Twenty-eight of the strains in Table 1 were non-lactose fermenting, although the majority of EAggEC are lactose-fermenting (Barroe & Feltham, 2003; Scheutz & Strockbine, 2007). Lactose-fermenting strains are less likely to be sent to the

reference laboratory for confirmation or exclusion of *Shigella* species. Eight of the nine isolates submitted for confirmation or exclusion of VTEC O157 were non-sorbitol fermenting strains. Although there are no routine protocols for the detection of EAggEC in the diagnostic microbiology laboratory in the UK, the results from this study show that this group of putative pathogens may be the cause of IID, especially in cases associated with travel. Microbiology laboratories are increasing their repertoire of molecular assays, and the detection and identification of DEC in the routine diagnostic environment is now possible. Routine detection will help determine the true prevalence of DEC in the UK and identify possible sources of infection and associated risk factors.

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Table 1. Strains of DEC submitted to GIRU between April and October 2009 and archive strains of *E. coli* O151 and O62 from 2004 to 2008

Patient no.	DEC group	Serotype	Identification made by sender	Countries recently visited by patient	Lactose fermenting
IpaH 2009 isolates					
1	EIEC	O?:H-	<i>E. coli</i>	India	—
2	EIEC	O121:H-	<i>S. dysenteriae</i>	Egypt	—
3	EIEC	O121:H-	<i>S. dysenteriae</i>	Egypt	—
4	EIEC	O?:H30	<i>S. dysenteriae</i>	Kenya	—
5	EIEC	O121:H-	<i>S. dysenteriae</i>	Egypt	—
Aat 2009 isolates					
6	EAggEC	O151:H-	<i>Shigella</i> sp.	Egypt	—
7	EAggEC	O?:H32	VTEC	No data	+
8	EAggEC	O?:H5	Unable to identify	No data	—
9	EAggEC	O55	<i>E. coli</i>	No data	+
10	EAggEC	O151:H-	<i>S. boydii</i>	No data	—
11	EAggEC	O62:H30	<i>S. flexneri</i>	Egypt	—
12	EAggEC	O62:H30	VTEC	Uganda	—
13	EAggEC	O151:H-	VTEC	Egypt	—
14	EAggEC	O?:H11	<i>Shigella</i> sp.	No data	—
15	EAggEC	O151:H11	<i>S. boydii</i>	Egypt	—
16	EAggEC	O151:H-	<i>Shigella</i> sp.	Egypt	—
17	EAggEC	O151:H11	<i>Shigella</i> sp.	Pakistan	—
18	EAggEC	O151:H-	<i>Shigella</i> sp.	Egypt	—
19	EAggEC	O?:H12	VTEC	No data	+
20	EAggEC	O?:H12	VTEC	Egypt	+
Archive strains belonging to <i>E. coli</i> O151 and O62 between 2004 and 2008					
21	<i>E. coli</i>	O151:H-	VTEC	No data	+
22	EAggEC	O151:H11	<i>Shigella</i> sp.	Egypt	—
23	<i>E. coli</i>	O151:H-	<i>Shigella</i> sp.	Egypt	—
24	<i>E. coli</i>	O151:H-	<i>Shigella</i> sp.	Egypt	—
25	EAggEC	O151:H-	<i>Shigella</i> sp.	Turkey	—
26	<i>E. coli</i>	O151:H-	<i>S. flexneri</i>	Iraq	—
27	EAggEC	O151:H-	VTEC	Egypt	+
28	EAggEC	O151:H-	<i>S. boydii</i>	Egypt	—
29	EAggEC	O151:H-	<i>S. boydii</i>	No data	—
30	EAggEC	O62:H-	<i>Shigella</i> sp.	No data	—
31	EAggEC	O62:H30	<i>S. boydii</i>	No travel	—
32	EAggEC	O62	<i>Shigella</i> sp.	No travel	+
33	EAggEC	O62	<i>Shigella</i> sp.	No data	+
34	EAggEC	O62:H30	<i>E. coli</i>	No data	+
35	EAggEC	O62:H30	<i>S. boydii</i>	No data	—
36	EAggEC	O62	VTEC	Egypt	+
37	EAggEC	O62:H30	VTEC	Egypt	—
38	EAggEC	O62:H30	<i>S. flexneri</i>	No data	—

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