A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from cases of bloody diarrhoea, non-bloody diarrhoea and asymptomatic contacts

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Enrichment culture in modified buffered peptone water followed by immunomagnetic separation (IMS) with magnetic beads coated with an antibody against *Escherichia coli* O157 was compared with direct culture on cefixime rhamnose sorbitol MacConkey agar (CR-SMAC) and cefixime tellurite sorbitol MacConkey agar (CT-SMAC) for the isolation of *E. coli* O157 from human faeces. In total, 690 samples were examined; *E. coli* O157 was isolated from 25 samples by IMS but from only 15 and 12 by direct culture on CT-SMAC and CR-SMAC, respectively. The difference in sensitivity of detection was at its most marked on screening repeat faecal samples from known cases and samples from asymptomatic contacts, when of 12 strains of *E. coli* O157 isolated by IMS, only five were isolated by direct culture. IMS is a sensitive and simple technique for the isolation of *E. coli* O157 from human faecal samples and should prove useful in elucidating further the epidemiology of this micro-organism.

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) strains are now recognised as a major cause of both haemorrhagic colitis (HC) and the haemolytic uraemic syndrome (HUS), conditions with high morbidity and mortality. In the UK, verocytotoxin-producing (VT⁺) strains of *E. coli* O157, the most common serogroup associated with illness in man, have been isolated from cattle [1–3], and beef, beef products, milk and milk products have been identified as sources of human infection [2–4]. Person-to-person transmission has also been reported [5–7], but the relative importance of this route of spread is unclear. In view of the potential severity of infections caused by VT⁺ *E. coli* O157 strains it is essential that the most sensitive methods available are used in studies designed to elucidate further the epidemiology of the micro-organism.

VT⁺ *E. coli* O157 strains do not ferment sorbitol whereas most other serogroups of *E. coli* do, so that sorbitol MacConkey (SMAC) agar has proved useful for their isolation [8]. Improvements to SMAC medium [9, 10] have resulted in increased sensitivity

of isolation of *E. coli* O157 strains from faecal samples but efficient selective enrichment media have not been described. Enrichment culture in buffered peptone water with antibiotics, with subculture to SMAC supplemented with cefixime 0.05 mg/L and rhamnose 0.5% w/v (CR-SMAC) [9] has been used previously for isolating *E. coli* O157 strains from beef carcasses [2] but was of low sensitivity, detecting an initial inoculum of only 2000 cfu/10 g of beef. Inclusion of an immunomagnetic separation (IMS) step in the isolation procedure enhanced this sensitivity at least 100-fold to a detection limit of 2–20 cfu/10 g of beef [11]. When IMS was used to examine bovine faecal suspensions inoculated with 12 different strains of *E. coli* O157, it was *c.* 100-fold more sensitive in detecting the organism than direct culture [12], an increase in sensitivity that was borne out in a field study of a dairy herd, when of 84 strains of *E. coli* O157 isolated from 1024 rectal swabs taken from cattle over a 4-month period, 23 were isolated by both direct culture and IMS, but 61 were isolated by IMS only [12]. The aim of this study was to evaluate the use of enrichment culture in modified buffered peptone water [2] followed by IMS with magnetic beads coated with an antibody to *E. coli* O157 for the isolation of *E. coli* O157 from faecal samples from cases of acute bloody and non-bloody diarrhoea and from repeat samples and asymptomatic contacts of these cases.

Materials and methods

Selection of faecal samples

The study was conducted between 1 July 1994 and 30 June 1995. Faecal samples from patients with bloody diarrhoea or non-bloody diarrhoea were selected from those submitted to the laboratory by general practitioners and local hospitals on the basis of information on the request form, or because blood was present in the sample. Wherever possible, samples from asymptomatic contacts and repeat samples from positive patients were collected by Sheffield and Rotherham Environmental Health Departments and submitted to Sheffield Public Health Laboratory. Repeat samples were collected at intervals ranging from 1 week to 3 months after the date of onset of initial symptoms.

Direct culture of E. coli O157

Approximate 10- μ l volumes of faecal samples were inoculated directly on to cefixime rhamnose sorbitol MacConkey (CR-SMAC) medium [9] and cefixime tellurite sorbitol MacConkey (CT-SMAC) medium [10]. After overnight incubation at 37°C, sorbitol and rhamnose non-fermenting colonies from CR-SMAC and sorbitol non-fermenting colonies from CT-SMAC were tested for agglutination with a latex test kit (Oxoid, DR622) for detecting *E. coli* O157. Isolates that gave positive results with this test were further characterised as described below.

Immunomagnetic separation of E. coli O157

After direct culture had been made, c. 0.5 g of faecal sample was placed in 5 ml of buffered peptone water (Oxoid, CM509) supplemented with vancomycin 8 mg/L, cefixime 0.05 mg/L and cefsulodin 10 mg/L (BPW-VCC), to inhibit the growth of gram-positive organisms, aeromonads and *Proteus* spp. [2]. After vortex mixing, broths were incubated at 37°C for 6 h and 1 ml of broth was then added to 20 μ l of magnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Oslo) in a 1.5-ml microcentrifuge tube. The beads were suspended evenly in the broth culture by vortex mixing and were placed in a rotating mixer so that they were mixed by inversion every 2–3 s for 30 min at ambient temperature. Tubes were placed in a magnetic separator rack (MPC-10, Dynal) and the magnets were placed in position and left for 5 min. The culture supernate was removed by aspiration with a pasteur pipette, the magnetic slide was removed from the rack, the beads were washed by resuspension in 1 ml of PBS, pH 7.2, with Tween-20 0.05% v/v (PBST) and the magnetic slide was replaced for 2 min. The beads were washed in PBST in this way once more, the magnetic slide was replaced for 2 min, the supernate was removed and the beads were resuspended in c. 25 μ l of PBS. Beads were

inoculated on to CT-SMAC medium and incubated overnight at 37°C. Sorbitol non-fermenting colonies were examined as above. Sorbitol non-fermenting colonies that did not agglutinate with the latex test kit for *E. coli* O157 were identified by a standard series of biochemical tests [1].

Quantification of E. coli O157

When possible, quantification of the *E. coli* O157 isolates by IMS but not by direct culture was performed by adding 5 $\times$ 1 g, 5 $\times$ 0.1 g and 5 $\times$ 0.01 g of faeces to 10-ml volumes of BPW-VCC, vortex mixing, incubating at 37°C for 6 h and performing IMS and examination of sorbitol non-fermenting colonies as above. The most probable number of *E. coli* O157 was then estimated by standard methods [13].

Identification

As described previously [1], isolates that gave a positive latex test result were confirmed as *E. coli* by biochemical tests and confirmed as serogroup O157 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, 61 Colindale Avenue, London).

Verocytotoxin production

Toxigenicity was determined by Vero cell culture assay [1]. Toxin type was determined by specific hybridisation with DNA probes for the VT₁ and VT₂ genes. DNA specific for the A cistrons of the VT₁ and VT₂ genes was prepared by the polymerase chain reaction, random-prime labelled with digoxigenin-11-dUTP, and used in colony hybridisation reactions as described previously [2, 14]. Known VT₁⁺, VT₂⁺ and VT⁻ strains were included as controls in each batch of tests.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method [15] and were separated by submerged gel electrophoresis in Tris-acetate-EDTA buffer with agarose 1%, stained by ethidium bromide and visualised on an ultraviolet transilluminator. A control *E. coli* K-12 strain (NCTC 50192-39R861) harbouring plasmids of 148, 63.4, 36 and 6.9 kb was included with each batch of tests. For this control strain, the log₁₀ of plasmid size was plotted against distance migrated through the agarose gel and approximate sizes of plasmids from strains of *E. coli* O157 were estimated from this graph.

Phage typing

All *E. coli* O157 isolates were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory.

Results

E. coli O157 was isolated from 10 (3.7%) of 272 patients with acute bloody diarrhoea, three (0.8%) of 375 patients with acute non-bloody diarrhoea, eight (42%) of 19 repeat samples from these patients and four (16.7%) of 24 asymptomatic contacts. Results are shown in detail in Table 1. Sufficient sample to enable quantification of *E. coli* O157 was available from only two asymptomatic contacts; in both cases numbers of *E. coli* O157 were < 10 cfu/g of faeces.

The identities of 148 sorbitol non-fermenting organisms which were not *E. coli* O157, but which apparently adhered to the magnetic beads, are shown in Table 2. The two most common groups were *E. coli* strains of other serogroups (32.4%) and *Proteus* spp. (19.6%).

Characteristics of the 17 primary isolates of *E. coli* O157 are shown in Table 3. Only one strain was non-toxicogenic and gave negative results in both cell culture and DNA hybridisation assays; this strain was isolated only by IMS and was from a patient with acute non-bloody diarrhoea. Two strains reacted with the phage set but did not conform to recognised phage types. All strains harboured the 92-kb plasmid which is a common characteristic of VTEC.

Table 2. Sorbitol non-fermenting organisms adhering to *E. coli* O157 Dynabeads

Species	Number (%)
<i>E. coli</i> , not O157	48 (32.4)
<i>Hafnia alvei</i>	17 (11.5)
<i>Morganella morganii</i>	13 (8.8)
<i>Proteus mirabilis</i>	11 (7.4)
<i>Proteus vulgaris</i>	5 (3.4)
<i>Klebsiella ozaenae</i>	5 (3.4)
<i>Providencia alcalifaciens</i>	4 (2.7)
Gram-negative organisms identified to genus level	12 (8.1)
Gram-negative organisms not identified to genus level	33 (22.3)
Total	148

Discussion

Cattle appear to be a major reservoir of VT⁺ *E. coli* O157 strains and the organism has been isolated from apparently healthy animals during investigations of sources of human infection in Canada [16], the USA [17–19] and England [1–3]; the enhanced sensitivity of IMS for detecting strains of *E. coli* O157 in food, milk and bovine faecal samples [3, 11, 12] has proved useful in epidemiological investigations in the Sheffield area [3, 20]. However, although primarily a foodborne

Table 1. Comparison of IMS with direct culture on CR-SMAC and CT-SMAC for isolation of *E. coli* O157 from human faecal samples

Patient category	Number of specimens	Number of cultures requiring investigation of SNF colonies from			Number of <i>E. coli</i> O157 isolates obtained by		
		CR-SMAC	CT-SMAC	IMS	CR-SMAC	CT-SMAC	IMS
Bloody diarrhoea	272	9	14	45	8	9	10
Acute non-bloody diarrhoea	375	10	33	116	1	1	3
Follow-up samples	19	3	4	8	3	4	8
Asymptomatic contacts	24	0	1	4	0	1	4
Total	690	22	52	173	12	15	25

SNF, sorbitol non-fermenting.

Table 3. Characteristics of 17 strains of *E. coli* O157 isolated from human faeces by IMS

Phage type	Vero cell assay for VT	DNA probes for			Number of isolates
		VT ₁	VT ₂	Plasmids (kb)	
1	+	+	+	92, 6.6	1
2	+	–	+	92	7
	+	–	+	92, 7.5, 5.7	2
	+	–	+	92, 85, 75	1
	+	–	+	92, 60	1
	+	–	+	92, 8.3	1
4	+	+	+	92, 6.3	1
32	–	–	–	92	1
RDNC	+	–	+	92, 69, 2	1
	+	–	+	92, 5, 2	1

RDNC, reacted with the phage set but did not conform to a recognised phage type.

illness, person-to-person transmission has been described, mainly in institutional and family outbreaks [5–7]. Reports vary as to whether person-to-person transmission is facilitated by prolonged excretion of *E. coli* O157. According to Tarr *et al.* [21], all those infected with VT⁺ *E. coli* O157 had positive faecal cultures up to 2 days after onset of illness, but at ≥ 7 days after onset, only 30% were positive. In contrast, Belongia *et al.* [5] found excretion of the organism for up to 62 days and Gransden *et al.* [22] for up to 28 days. The latter group speculated that prolonged excretion may aid the spread of *E. coli* O157, particularly among children. In the present study, eight of 19 repeat samples from previously positive patients were positive for *E. coli* O157 but only three and four were positive by direct culture on CR-SMAC and CT-SMAC, respectively. These included a 2-year-old child who was shown to be still excreting the organism 3 months after initial onset of symptoms. The role of asymptomatic carriers in the spread of *E. coli* O157 infection is not known, but in this study four asymptomatic contacts of cases were found to be positive by IMS – only one of these was detected by direct culture on CT-SMAC but not on CR-SMAC. In two asymptomatic contacts who were positive by IMS only, the numbers of *E. coli* O157 excreted in the faeces were low (<10 cfu/g) and this probably explains their detection only by the IMS technique. However, given the presumed low infective dose of the organism, such carriers may be important in the spread of infection. Therefore, IMS is a useful technique for the monitoring of human infections by *E. coli* O157 and is more sensitive than direct culture.

All strains isolated by IMS and direct culture were typical of those isolated in the Sheffield area in previous studies. One strain was non-toxigenic, phage type 32 and harboured plasmids of 92 and 2 kb. This strain was isolated from a case of acute non-bloody diarrhoea and although it has been isolated previously from cattle [2], this is the first instance of its isolation from a human source; its significance in relation to the patient's illness is unclear.

The main problem found with the IMS technique was the number of sorbitol non-fermenting micro-organisms other than *E. coli* O157 that adhered non-specifically to the magnetic beads (Table 2). At least 10 different types of such organisms were found in the present study with the two most common groups being *E. coli* strains of other serogroups (32.4%) and *Proteus* spp. (19.6%); these organisms have also been a problem with direct culture techniques [9]. Although this did not detract from an overall 67% increase in positive samples detected by IMS over direct culture, it did increase the technical time required for the investigations and further work is required to address this problem. IMS is rapid, technically simple and sensitive and should be useful for the monitoring of human infections with *E. coli* O157.

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