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Reliability of CHROMagar® O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups

K.A. Bettelheim
VIDRL, Fairfield, Melbourne, Australia

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K.A. BETTELHEIM. 1998. CHROMagar® O157 is designed for the rapid isolation and identification of enterohaemorrhagic *Escherichia coli* (EHEC), particularly O157, characterized by pink colonies. Five hundred and eighty-five *E. coli* strains, including O157, O111 and O113 serogroups, from many sources were examined on CHROMagar® O157. Enterohaemorrhagic *E. coli* O157 could readily be isolated and recognized uniquely by typical pink colonies. Some other EHEC also produced pink colonies, whereas O113 and many other EHEC strains were blue and indistinguishable from Shiga-like toxin-negative strains of *E. coli*.

INTRODUCTION

Enterohaemorrhagic *Escherichia coli* (EHEC) are being increasingly associated with both human and animal infection. An important component of their virulence is the production of one or both of the Shiga-like toxins (SLT), I or II. Strains belonging to serogroup O157, particularly serotypes O157:H7 and O157:H−, have been predominantly associated with many outbreaks and sporadic cases throughout the world since their first description in 1982 (Riley *et al.* 1983) and have been incriminated in the major outbreak involving four western states of the USA and affecting nearly 600 individuals and causing four deaths (Centers for Disease Control and Prevention 1993). More recently there was an outbreak in Japan involving nearly 10 000 cases in 1996 (Watanabe *et al.* 1996) and outbreaks have since been regularly reported, including in Scotland in 1996/7, the USA in 1997 and around the world. However, other serotypes have also been described as causing both sporadic cases as well as outbreaks, most notably O111 in Italy (Caprioli *et al.* 1994), Japan (Kudoh *et al.* 1994) and Australia (Cameron *et al.* 1995). A review of the literature indicates that there may be at least 100 EHEC serotypes present around the world, many of which have been associated with human disease.

Enterohaemorrhagic *E. coli* strains belonging to serogroup O157 have been shown generally to lack the ability to ferment sorbitol. The development of the sorbitol MacConkey agar (SMAC) has enabled many laboratories to isolate these organisms (March and Ratnam 1986) and this medium has been extensively used over the last few years. Strains of EHEC O157:H7 have been described which will ferment sorbitol (Gunzer *et al.* 1992), and EHEC of other serogroups generally ferment sorbitol. Also strains of *E. coli* O157 have been isolated which do not ferment sorbitol but are also not verocytotoxigenic and have not been associated with human disease (Pearce *et al.* 1994).

Beutin *et al.* (1989) noted a close association between the production of SLT and enterohaemolysin among EHEC strains belonging to serogroups O157, as well other serogroups including O26, O111 and O116. These studies were extended (Bettelheim 1995) and confirmed the strong relationship between SLT and enterohaemolysin production, on the basis of which a rapid method of identifying EHEC was proposed. The disadvantage of this method is that it requires two plates and cannot easily be applied for the primary isolation of EHEC, although it is a reliable, rapid and inexpensive confirmatory test.

A new medium, CHROMagar® O157, has recently been brought onto the market by CHROMagar (Paris, France), which should enhance the primary isolation of EHEC from food and faecal cultures on primary isolation. According to the manufacturers, strains of EHEC O157:H7 form characteristic pink to purple colonies, while commensal *E. coli* form blue or rarely colourless colonies.

Correspondence to: K.A. Bettelheim, VIDRL, Locked Bag 815, Carlton South, Victoria 3053, Australia.

MATERIALS AND METHODS

Strains investigated

In this study strains of EHEC as well as other *E. coli* obtained from a variety of sources from around the world, including Africa, Asia, Australia, Europe and North America, were used. They were all from the culture collection in the VIDRL (Fairfield, Victoria, Australia) and had been collected over the years as part of a number of investigations. They were all stored on Dorset egg medium. While many of the EHEC had been submitted for identification from cases of haemorrhagic colitis or haemolytic uraemic syndrome (HUS), strains were also requested from laboratories around the world. A number of strains which had been submitted as part of environmental studies as well as requested strains were included. Also selected were strains isolated from healthy children. All the strains were characterized as being *E. coli* on the basis of their characteristic reaction in triple sugar iron agar, their ability to produce indole and to split ortho-nitro-phenyl galactoside and their inability to split urea. They were serotyped for their 'O' and 'H' antigens (Bettelheim and Thompson 1987; Chandler and Bettelheim 1974). They were tested for their ability to produce SLT by the cell assay using vero cells (Konowalchuk *et al.* 1977). All strains were also tested by the ELISA technique described by Acheson *et al.* (1990) using sheep hydatid cyst fluid (obtained from Dr M. Lightowlers, School of Veterinary Science, University of Melbourne, Veterinary Clinical Centre, Werribbee, Victoria, Australia) to capture toxin. Monoclonal antibodies 13C4, which is specific for SLT I, obtained from the American Type Culture Collection (No. CRL 1794) (O'Brien *et al.* 1982) and 11E10, which produces antibody specific for SLT II (obtained from Dr A. O'Brien, Armed Services University of the Health Sciences, Bethesda, Maryland, USA) (Perera *et al.* 1988), were used to confirm presence of the toxin(s). In many cases the presence of the genes coding for the SLT was tested for by the polymerase chain reaction technique, currently being developed in our laboratory. Generally full agreement between the tests for presence of SLT by the three methods was achieved. All the strains were tested for their ability to produce enterohaemolysin (Bettelheim 1995).

Testing of CHROMagar® O157

For the tests the CHROMagar® O157 was prepared according to the manufacturer's instructions. All strains were cultivated on Columbia Agar (Oxoid) overnight at 37 °C to confirm purity. They were then spread onto CHROMagar® O157, incubated overnight at 37 °C and examined for appearance of the colonies, with particular emphasis on their colour.

RESULTS

The results of testing the strains and the colours obtained are given in Table 1.

DISCUSSION

Of the strains of O157, which included both O157:H7 as well as O157:H−, there was a very strong correlation between SLT production and the formation of pink-coloured colonies. The three non-SLT-producing O157 strains were all environmental. They gave the same characteristic biochemical reactions as the typical SLT-producing O157 strains. It is possible to speculate that they may well be capable of acquiring the extrachromosomal generic elements determining SLT production and the other virulence factors required for human infection. One of the two SLT-producing O157 strains which did not give pink colonies on the CHROMagar® O157 was a sorbitol-fermenting strain which formed blue colonies. This strain may well have other biochemical anomalies, which is why it did not give the characteristic pink colour. It would also have been missed on the SMAC agar, but it did produce enterohaemolysin.

With the non-O157 strains the situation is more complex. Of the O111 strains, which have been associated with outbreaks in Italy (Caprioli *et al.* 1994), Japan (Kudoch *et al.* 1994) and Australia (Cameron *et al.* 1995), there is a reasonably good correlation between production of the pink to purple colour and SLT production. Strains from both the Australian and Italian outbreaks could have been identified using CHROMagar® O157.

However, most other EHEC serotypes consistently grew as blue colonies. This was particularly noted with strains of O113:H21 which have been isolated from cases of HUS in many parts of the world (Karmali 1989; Bettelheim *et al.* 1990). The strains belonging to this serotype would not be selected using this agar, if one only concentrated on pink colonies. They appear no different from other typical non-toxigenic serotypes. This is particularly significant, because many toxigenic strains of O113:H21 isolated from human cases including HUS also do not produce enterohaemolysin. Most other EHEC serotypes, including O5:H−, O26:H11; O48:H21; O91:H−; O111:H2; O111:H8; O111:H− and O128:H2, would also have been missed.

Microbiologists should be aware that a certain difficulty may be encountered with the SLT-negative strains, which give pink to purple colonies, of which there were 20 (6·25%) in this selection of 320 SLT-negative strains. They did include some strains belonging to serogroups O26, O111 and O157 which could potentially be considered as EHEC.

The situation is thus that virtually all SLT-producing strains of *E. coli* O157 (both H7 and H−) give characteristic pink to purple colonies on CHROMagar® O157; once seen they would not be mistaken. A few other EHEC, particularly O111, also give pink to purple colonies and would not be missed.

The CHROMagar® O157 is a useful addition to the battery of tests for the isolation and identification of EHEC, an every-increasing problem. While the ability to detect SLT-

Table 1 Shiga-like toxin (SLT) production of different O serogroups related to colour of colonies on CHROMagar® O157

SLT+/pink		SLT+/blue		SLT−/pink		SLT−/blue					
Serogroups	No.	Serogroups	No.	Serogroups	No.	Serogroups	No.	Serogroups	No.	Serogroups	No.
O26	1	O5	13	O1	2	O1	10	O30	2	O106	3
O88	2	O6	2	O26	1	O2	14	O32	2	O107	1
O111	26	O9	1	O60	1	O3	2	O33	2	O111	36
O157	90	O26	13	O111	4	O4	18	O34	1	O112ac	1
Ont	2	O28	1	O113	1	O5	2	O35	1	O113	2
OR	2	O45	1	O119	1	O6	17	O36	2	O116	1
		O48	1	O125	4	O7	5	O37	1	O119	2
		O55	2	O148	1	O8	5	O38	1	O125	5
		O69	1	O157	3	O9	1	O39	2	O126	7
		O71	1	O90/127	1	O10	2	O40	1	O128	8
		O75	3	Ont	1	O11	3	O41	2	O128	3
		O81	2			O12	1	O42	1	O130	1
		O91	22			O13	1	O43	1	O135	1
		O98	3			O14	1	O44	1	O141	2
		O104	3			O15	5	O45	2	O149	1
		O111	6			O16	3	O46	2	O153	2
		O112ab	2			O17	2	O49	1	O154	2
		O113	16			O18a,b	1	O51	2	O155	1
		O116	2			O18a,c	3	O52	2	O156	3
		O123	7			O19	1	O55	4	O157	10
		O130	1			O20	1	O62	1	O160	1
		O137	1			O21	3	O75	7	O161	1
		O145	1			O22	2	O77	2	O162	1
		O146	2			O23	1	O78	2	O163	1
		O153	3			O24	1	O80	1	O164	2
		O156	1			O25	14	O81	1	O21/83	1
		O157	2			O26	2	O84	1	O90/127	1
		O163	3			O27	1	O86	3	Ont	16
		O168	2			O28	1	O88	2	OR	6
		O172	1			O29	1	O99	1		
		Ont	20								
		Or	3								
Total	123	Total	142	Total	20					Total	300

producing strains of EHEC O157 rapidly and easily is an important addition to the ability to identify these emerging pathogens, it is also important that the CHROMagar® O157 will readily detect EHEC strains of O111. It is important to note that most of the other EHEC serotypes will not be detected. The CHROMagar® O157 is only claimed by the manufacturer to be very specific for EHEC O157 strains. The results presented generally confirm these claims. It is a particular bonus that many EHEC O111 strains also produce pink to purple colonies. If SLT-producing *E. coli* are suspected, this medium would grow them and they could then be tested by other means for SLT production.

While there is a strong correlation between outbreaks of HUS and isolation of serotypes O157:H7 or O157:H−, it should not be forgotten that many other serotypes, including O26, O91, O111 and O113, may also cause similar infections and they may in fact be present in addition to O157 (Goldwater and Bettelheim 1996). It is essential to realize that an isolate should not be considered a pathogen purely because of some biochemical characteristic unrelated to pathogenicity,

such as production of pink to purple colonies on certain media or inability to ferment sorbitol.

REFERENCES

Acheson, D.W.K., Keusch, G.T., Lightowlers, M. and Donohue-Rolfe, A. (1990) Enzyme linked immunosorbent assay for Shiga toxin and Shiga-like toxin II using P1 glycoprotein from hydatid cysts. *Journal of Infectious Diseases* 127, 1145–1150.

Bettelheim, K.A. (1995) Identification of enterohaemorrhagic *Escherichia coli* by means of their production of enterohaemolysin. *Journal of Applied Bacteriology* 79, 176–180.

Bettelheim, K.A., Brown, J.E., Lolekha, S. and Echeverria, P. (1990) Serotypes of *Escherichia coli* that hybridized with DNA probes for genes encoding Shiga-like toxin I, Shiga-like toxin II, and serogroup O157 enterohaemorrhagic *E. coli* fimbriae isolated from adults with diarrhoea in Thailand. *Journal of Clinical Microbiology* 28, 293–295.

Bettelheim, K.A. and Thompson, C.J. (1987) New method of serotyping *Escherichia coli*: implementation and verification. *Journal of Clinical Microbiology* 25, 781–786.

Beutin, L., Montenegro, M.A., Ørskov, I., Ørskov, F., Prada, J., Zimmermann, S. and Stephan, R. (1989) Close association of verocytotoxin (shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *Journal of Clinical Microbiology* 27, 2559–2564.

Cameron, S., Walker, C., Beers, M., Rose, N. and Anear, E. (1995) Enterohaemorrhagic *Escherichia coli* outbreak in South Australia associated with consumption of mettwurst. *Communicable Disease Intelligence* 19, 70–71.

Caprioli, A., Luzzi, I., Rosmini, F., Resti, C., Edefonti, A., Perfumo, F., Farina, C., Goglio, A., Gianviti, A. and Rizzoni, G. (1994) Community wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. *Journal of Infectious Diseases* 169, 208–211.

Centers for Disease Control and Prevention (1993) Update: multistate outbreak of *Escherichia coli* O157 infections from hamburgers—Western United States, 1992–1993. *Morbidity and Mortality Weekly Report* 42, 258–263.

Chandler, M.E. and Bettelheim, K.A. (1974) A rapid method of identifying *Escherichia coli* "H" antigens. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene I. Abteilung Originale A* 129, 74–79.

Goldwater, P.N. and Bettelheim, K.A. (1996) An outbreak of hemolytic uremic syndrome due to *Escherichia coli* O157:H7: or was it? *Emerging Infectious Diseases* 2, 153–154.

Gunzer, F., Böhm, H., Russmann, H., Bitzan, M., Aleksic, S. and Karch, H. (1992) Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. *Journal of Clinical Microbiology* 30, 1807–1810.

Karmali, M.A. (1989) Infection by Verocytotoxin-producing *Escherichia coli*. *Clinical Microbiology Reviews* 2, 15–38.

Konowalchuk, J., Speirs, J.L. and Stavric, S. (1977) Vero response to a cytotoxin of *Escherichia coli*. *Infection and Immunity* 18, 775–779.

Kudoh, Y., Kai, A., Obata, H., Kusonoki, J., Monma, C., Shimgaki, M., Yanagawa, Y., Yamada, S., Matsushita, S., Itoh, T. and Otah, K. (1994) Epidemiological surveys on verotoxin-producing *Escherichia coli* infections in Japan. In *Recent Advances in Verocytotoxin-producing* Escherichia coli *Infections*. ed. Karmali, M.A. and Goglio, A.G. pp. 53–56. Amsterdam: Elsevier Science B.V.

March, S. and Ratnam, S. (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with haemorrhagic colitis. *Journal of Clinical Microbiology* 23, 869–872.

O'Brien, A.D., LaVeck, G.D., Thompson, M.R. and Formal, S.B. (1982) Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. *Journal of Infectious Diseases* 146, 763–769.

Pearce, J.L., Bettelheim, K.A., Reed, B., Cranwell, P.D. and Luke, R.J.K. (1994) Isolation of sorbitol non-fermenting, motile *Escherichia coli* O157 from scouring piglets. *Australian Veterinary Journal* 71, 156.

Perera, L.P., Marques, L.R.M. and O'Brien, A.D. (1988) Isolation and characterisation of monoclonal antibodies to Shiga-like toxin II of enterohaemorrhagic *Escherichia coli* and use of the monoclonal antibodies in a colony enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* 26, 2127–2131.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, G.J., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A. and Cohen, M.L. (1983) Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* 308, 681–685.

Watanabe, H., Wada, A., Inagaki, Y., Itoh, K.-I. and Tamura, K. (1996) Outbreaks of enterohaemorrhagic *Escherichia coli* O157:H7 infection by two different genotype strains in Japan, 1996. *Lancet* 248, 831–832.