

An improved selective medium for the isolation of *Escherichia coli* O157

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Summary. Sorbitol-MacConkey medium has become widely used for the isolation of verotoxigenic (VT⁺) *Escherichia coli* O157. However, many organisms other than VT⁺ *E. coli* O157, especially other serogroups of *E. coli* and *Proteus* spp., may not ferment sorbitol, and thus may be confused initially with VT⁺ *E. coli* O157. Rhamnose is not fermented by VT⁺ *E. coli* O157, but is by most sorbitol non-fermenting *E. coli* of other serogroups. Cefixime is a cephalosporin antibiotic that is more active against *Proteus* spp. than against *E. coli*. Inclusion of rhamnose and cefixime in sorbitol-MacConkey agar improves its selectivity for the isolation of VT⁺ *E. coli* O157.

Introduction

Strains of *Escherichia coli* that produce a potent cytotoxin active against cultured Vero cells are now recognised as important pathogens of man.¹ These verotoxigenic *E. coli* (VTEC) have been associated with outbreaks and sporadic cases of haemorrhagic colitis (HC) in North America^{2,3} and in England,^{4,5} and with sporadic cases of haemolytic-uraemic syndrome (HUS) in Canada^{6,7} and in England.^{5,8} Both HC and HUS have been associated with high morbidity and mortality.⁹

Beef products and untreated milk have both been suggested as possible sources of VTEC for man.^{2,10} Verotoxin-producing (VT⁺) *E. coli* O157, the most common serogroup of VTEC isolated from man, has been isolated from cattle,⁵ although the route of transmission from cattle to man was not established.¹¹ Person-to-person transmission of VT⁺ *E. coli* O157 has also been documented.^{12,13}

Strains of VT⁺ *E. coli* do not ferment sorbitol, and sorbitol-MacConkey (SMAC) medium has been described for their selective culture.¹⁴ This medium is now widely used in clinical diagnostic laboratories. However, some *E. coli* strains of serogroups other than O157 are also sorbitol non-fermenters (NSF), as are members of several other genera found frequently in faecal samples, especially *Proteus* spp.

All VT⁺ *E. coli* O157 isolated hitherto in the Sheffield area, and strains of VT⁺ *E. coli* O157 sent to us from elsewhere, have given identical results on biochemical testing.^{5,11} All isolates failed to ferment sorbitol, inositol, adonitol, rhamnose, cellobiose, sorbose and salicin. Biochemical tests on organisms isolated on SMAC from human and animal faeces

during 1987–1990, showed that 60% of NSF *E. coli* of serogroups other than O157 fermented rhamnose (Sheffield PHL—unpublished data). About 15% of NSF organisms isolated were *Proteus* spp. Cefixime is a cephalosporin antibiotic that is more active against *Proteus* spp. than against *E. coli*.¹⁵

The aim of this study was to determine whether inclusion of rhamnose and cefixime would improve SMAC medium for the isolation of VT⁺ *E. coli* O157.

Materials and methods

Cefixime MICs

Cefixime powder was a gift from Cyanamid (Gosport, Hants) and was prepared initially as a solution of 4 g/L in ethanol, as advised by the manufacturers. Doubling dilutions of cefixime, range, 4–0.002 mg/L, were prepared in 100- μ l volumes in sterile microtitration plates (Sterilin), with MacConkey Broth (Oxoid, CM5a) with glucose 1% as diluent. Isolates to be tested were grown in nutrient broth at 37°C for 3 h and these cultures were diluted 1 in 100 in diluent as above to prepare an inoculum of *c.* 10⁵ organisms/ml; 100 μ l of inoculum were added to each dilution of cefixime; after mixing, the plates were incubated at 37°C overnight. The lowest dilution of cefixime that inhibited growth (no turbidity or colour change) was recorded as the MIC. MICs were determined for 98 strains of VT⁺ *E. coli* O157 and 22 strains of *Proteus* spp. previously isolated from faecal samples.

Rhamnose fermentation

SMAC Medium (Oxoid, CM813) was prepared with added rhamnose, in a series of concentrations,

0.1–1% in 0.1% steps. Control organisms (VT⁺ *E. coli* O157 sorbitol-negative and rhamnose-negative, and *E. coli* O26 sorbitol-negative and rhamnose-positive) were grown on each plate to determine the lowest concentration of rhamnose that produced an easily observed colour change when fermented.

Cefixime-rhamnose-sorbitol-MacConkey (CR-SMAC) medium

SMAC medium was prepared with added cefixime 0.05 mg/L and rhamnose 0.5%. The medium was tested in parallel with SMAC for ability to support the growth of: VT⁺ *E. coli* O157 isolated in Sheffield (84 strains), VT⁺ *E. coli* O157 from diverse locations within the UK (96), *Proteus* spp. (34), NSF *E. coli* of serogroups other than O157 (27), *Morganella morganii* (16), *Providencia* spp. (15) and *E. hermannii* (2). The test strains were inoculated on to each medium and the plates were incubated at 37°C overnight. Growth and rhamnose fermentation were recorded. VT⁺ *E. coli* O157 strains were checked for latex-test reactivity from CR-SMAC by a previously described method.¹⁶

Comparison of SMAC and CR-SMAC in routine diagnostic tests

Selection of patients. Patients studied were those with acute diarrhoea of unknown aetiology. Faecal samples were sent from General Practices, the Communicable Diseases Unit of Lodge Moor Hospital, Sheffield, and other hospitals in the Sheffield area. All samples submitted during August and September 1990 were examined.

Examination of faecal samples. Samples were inoculated on to SMAC and CR-SMAC media and the plates were incubated at 37°C overnight. Each colonial type of non-fermenter was identified by a series of biochemical tests⁵ and a latex test¹⁶ for *E. coli* O157. If faecal samples were from patients with bloody diarrhoea, HC or HUS, and, therefore, more likely to contain VT⁺ *E. coli* O157,⁵ six non-fermenting colonies were examined from each medium.

Results

Cefixime MICs

Cefixime MICs for VT⁺ *E. coli* O157 were in the range 0.125–0.5 mg/L, and for *Proteus* spp. 0.004–0.016 mg/L. Table I shows further details of MIC results.

Rhamnose fermentation

An easily observed colour change as a result of fermentation was obtained only with rhamnose concentrations above 0.4%.

Table I. Cefixime MICs for VT⁺ *E. coli* O157 and *Proteus* spp.

MIC (mg/L)	Number of strains of	
	VT ⁺ <i>E. coli</i> O157 (n=98)	<i>Proteus</i> spp. (n=22)
0.5	12	—
0.25	76	—
0.125	10	—
0.06	—	—
0.03	—	—
0.016	—	9
0.008	—	10
0.004	—	3

Growth on CR-SMAC

All VT⁺ *E. coli* O157 test strains grew on CR-SMAC as non-fermenting colonies, and all gave positive latex test results. All strains of *Proteus* spp. were inhibited by CR-SMAC. Of 27 strains of NSF *E. coli* of serogroups other than O157, 25 grew on CR-SMAC and of these 23 fermented rhamnose. Fourteen of 16 *M. morganii* strains grew on the medium, as did 11 of 15 strains of *Providencia* spp; however of these 11, 10 were of much reduced colony size when compared with growth on SMAC medium. Both strains of *E. hermannii* grew on CR-SMAC and fermented rhamnose.

Comparison of CR-SMAC and SMAC in routine diagnostic tests

During the study period, 1763 samples were inoculated on to both media. VT⁺ *E. coli* O157 was isolated from seven samples; six of these isolates grew equally well on both media, but in one instance, five of six NSF colonies from SMAC were *Hafnia* spp. and only one was VT⁺ *E. coli* O157, whereas non-fermenting colonies from CR-SMAC medium were all VT⁺ *E. coli* O157. From SMAC medium, 411 non-fermenting colony types were investigated from 397 samples: of these, 169 were NSF *E. coli* of serogroups other than O157, 46 were *Proteus* spp. and 196 were other NSF genera. From CR-SMAC medium, 178 non-fermenting colony types were investigated from 176 samples; of these 44 were NSF *E. coli* of serogroups other than O157, nine were *Proteus* spp. and 125 were other NSF genera. Thus 397 (22.5%) samples required further investigations of growth from SMAC medium, and with 14 of these samples investigation of more than one NSF colony type was required. From CR-SMAC, growth from 176 (10%) of samples required further investigation, and with two of these samples investigation of more than one colony type was required. Table II shows the results of the comparison in full.

Table II. Comparison of SMAC and CR-SMAC medium for primary isolation of VT⁺ *E. coli* O157

NSF organism isolated	Number of isolates obtained on	
	SMAC	CR-SMAC
VT ⁺ <i>E. coli</i> O157	7 (0.4)*	7 (0.4)
Other NF <i>E. coli</i>	169 (9.6)	44 (2.5)
<i>Proteus</i> spp.	46 (2.6)	9 (0.5)
<i>M. morgani</i>	33 (1.9)	37 (2.1)
<i>Hafnia</i> spp.	28 (1.6)	15 (0.9)
<i>Providencia</i> spp.	20 (1.1)	4 (0.2)
<i>Enterobacter</i> spp.	13 (0.7)	13 (0.7)
<i>Pseudomonas</i> spp.	12 (0.7)	12 (0.7)
<i>Aeromonas</i> spp.	7 (0.4)	7 (0.4)
Others	69 (3.9)	37 (2.1)
Total	411 (23.3)	178 (10.1)

*Numbers in parenthesis are the percentages of samples from which the organism was isolated.

Discussion

HC and HUS are serious infections affecting patients of all ages and of either sex.¹ Indeed, HUS, which is more common in young children, is now the single commonest cause of renal failure in children in North America and in England and Wales.^{1, 17} VTEC strains are now recognised as important causal agents of both these conditions.²⁻⁷ Unfortunately, in most cases, the source of these infections has not been elucidated, and the epidemiology of the infections remains unclear. The use of simpler methods for the isolation and identification of VT⁺ *E. coli* O157 would facilitate further epidemiological studies.

It has been reported that VT⁺ *E. coli* O157 strains do not ferment sorbitol, unlike most other *E. coli*.^{3, 18} Therefore, SMAC medium has become widely used for the isolation of VT⁺ *E. coli* O157.^{4, 5, 12, 19, 20} However, reports of its selectivity for VT⁺ *E. coli* O157 differ widely. Using SMAC, Smith *et al.*⁴ isolated VT⁺ *E. coli* O157 from 22% of samples examined, whereas Walker *et al.*²⁰ isolated these organisms from only 0.5%; this disparity is likely to reflect differences between highly selected samples sent to a reference laboratory and the routine examination of acute samples in a service laboratory. In their examination of faecal samples, several workers^{5, 20} have found NSF organisms other than *E. coli* O157 to be commoner than earlier reports from North America suggested. Walker *et al.*²⁰ found NSF organisms in 16% of faecal samples, most of which were NSF *E. coli* and *Proteus* spp. We have reported similar findings.⁵ In the present study, the results in table II show that in the examination of 1763 faecal samples, 411 organisms required further investigation from primary cultures on SMAC, whereas only 178 did so from CR-

SMAC medium. We believe that this would offer a significant saving of time and cost for a busy diagnostic laboratory. Seven isolates of VT⁺ *E. coli* O157 grew on both media. However, in one instance, when six non-fermenting colonies were examined from each medium, only one colony from SMAC was of VT⁺ *E. coli* O157, whereas all colonies from CR-SMAC were VT⁺ *E. coli* O157; in this case the organism could have easily been missed on SMAC medium. *E. hermannii* is a NSF organism which is isolated commonly from foodstuffs, and which may cross react antigenically with *E. coli* O157, causing false-positive results.^{21, 22} Although in the current study only two isolates of *E. hermannii* were examined both fermented rhamnose and, therefore, would be distinguished easily from VT⁺ *E. coli* O157 on CR-SMAC medium.

There are several alternatives to traditional techniques for the isolation and identification of VT⁺ *E. coli* O157. Genes coding for verotoxins 1 and 2 have been cloned and sequenced,²³⁻²⁶ and DNA probes produced from either labelled cloned fragments or synthetic oligonucleotides, have been described for detection of VTEC, including VT⁺ *E. coli* O157.^{27, 28} However, despite the sensitivity and specificity of these techniques, they are likely to be too expensive for, and beyond the scope of, most routine diagnostic laboratories. Serological confirmation of infection by detecting antibodies against purified LPS from VT⁺ *E. coli* O157 has been described,^{29, 30} but this test is one used only by reference laboratories. Detection of free faecal verotoxin by cell culture assay has been used with success as a diagnostic method,^{4, 5} but specific antitoxin is needed for confirmation of a positive result; this test is not yet available commercially.

In contrast to alternative methods, selective culture is simple, quick and inexpensive and isolates may be readily and reliably identified with a commercially available latex test kit.¹⁶ Isolation of the organism also allows typing by techniques such as phage typing³¹ and plasmid analysis, the latter technique having been shown to provide useful epidemiological information.¹¹

CR-SMAC performs better than SMAC for isolation of VT⁺ *E. coli* O157, but further evaluation is needed. Development of better selective media, and in particular enrichment culture, would facilitate further studies on the prevalence of this organism and the epidemiology of its infections.

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