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Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157

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Summary. Potassium tellurite was assessed for the selection of verocytotoxigenic (VT^+) *Escherichia coli* O157. MICs were higher for VT^+ *E. coli* O157 than for other strains of *E. coli* and for *Aeromonas* spp. MacConkey medium containing sorbitol, tellurite and cefixime (TC-SMAC) permitted the growth of VT^+ *E. coli* O157 and *Shigella sonnei* but partially or completely inhibited the growth of 67% of other strains of *E. coli* and all or most strains of other sorbitol-non-fermenting species tested. Of 391 rectal swabs from cattle screened on TC-SMAC medium, 26 yielded isolates of VT^+ *E. coli* O157 whereas sorbitol-MacConkey medium with cefixime and rhamnose yielded only nine isolates. Inclusion of potassium tellurite in sorbitol-MacConkey agar markedly increased the rate of isolation of VT^+ *E. coli* O157 from cattle rectal swabs and may do so for other types of specimen.

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 verocytotoxigenic *E. coli* (VTEC) strains have been associated with outbreaks and sporadic cases of haemorrhagic colitis (HC) in North America[2,3] and England[4,5] and with sporadic cases of haemolytic uraemic syndrome (HUS) in Canada[6,7] and England.[5,8] Both HC and HUS have been associated with high morbidity and mortality.[9]

Beef products and untreated milk have been suggested as possible sources of VTEC for man in North America.[2,10] Verocytotoxin-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] However, person-to-person transmission of VT^+ *E. coli* O157 has been documented.[12,13]

Strains of VT^+ *E. coli* O157 do not ferment sorbitol, and sorbitol-MacConkey (SMAC) medium was devised to facilitate their isolation.[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 faeces of man and cattle, especially *Proteus* spp. and *Aeromonas* spp. Media have been developed to decrease the number of NSF colonies that need to be screened during the attempted isolation of VT^+ *E. coli* O157. These include cefixime/rhamnose/sorbitol MacConkey (CR-SMAC) medium,[15] in which cefixime inhibits *Proteus* spp. at a concentration not inhibitory to *E. coli* and rhamnose is fermented by most NSF *E. coli* strains of serogroups other than O157, but not by VT^+ *E. coli* O157, and β-glucuronidase medium[16] in which colonies of VT^+ *E. coli* can be detected by their lack of fluorescence. However, no medium has yet been described that allows the growth of VT^+ *E. coli* O157 while inhibiting other strains of *E. coli*, although immunomagnetic separation[17] has been described as a method for the selective detection of *E. coli* O157 in food samples.

Tellurite has been used for 80 years for the isolation of pathogens including *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Vibrio cholerae* and *Shigella* spp.[18,19] Initial testing in this laboratory (PHLS unpublished data) suggested it had a selective activity for VT^+ *E. coli* O157. The aim of this study was to investigate this activity further and to develop and evaluate a selective medium for VT^+ *E. coli* O157 based on tellurite resistance.

Materials and methods

Bacterial strains

For MIC studies, 38 strains of VT^+ *E. coli* O157 from human faeces, 38 strains of VT^+ *E. coli* O157 from cattle rectal swabs, 25 strains of non-O157 *E. coli* isolated from food, six strains of NSF non-O157 *E. coli* from cattle faeces, 39 strains of non-O157 *E. coli* from human faeces, 24 strains of *Aeromonas* spp. from human and animal sources together with human isolates of *Proteus* spp. (20), *Morganella morganii* (10) and *Providencia* (10) were tested. Nine strains of sorbitol-fermenting *E. coli*, randomly isolated from cattle rectal swabs, and one bovine strain of VT^+ *E. coli* O157 were used in the study to resolve mixed

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cultures. To study growth on media selective for *E. coli* O157, 315 strains of VT$^+$ *E. coli* O157 from human faeces, 82 strains of VT$^+$ *E. coli* O157 from cattle rectal swabs, 100 strains of non-O157 *E. coli* randomly isolated from human faeces, 22 strains of *S. sonnei*, 103 strains of *Aeromonas* spp. from human and animal sources, 26 strains of *Proteus* spp., 10 strains of *Morganella morganii*, 10 strains of *Providencia* spp. and eight strains of *Plesiomonas* spp. were tested; all were human isolates.

Tellurite MICs

A range of dilutions of potassium tellurite was prepared in 100-μl volumes in 96-well microtitration plates (Sterilin), with MacConkey Broth (Oxoid CM5a) containing 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 to give an inoculum of *c.* 10^5 organisms/ml; 100 μl of inoculum were added to and mixed with each dilution of potassium tellurite (final concentrations 500–0·5 mg/L). The plates were incubated at 37°C overnight. The lowest dilution of potassium tellurite that inhibited growth (no turbidity or colour change) was recorded as the MIC.

Resolving mixed cultures

One colony from each of nine bovine strains of sorbitol-fermenting *E. coli* and one NSF colony of VT$^+$ *E. coli* O157 were suspended together in 1 ml of saline and 30 μl of this suspension was added to 5 ml of tryptone broth. A loopful of this was plated on to a series of SMAC plates containing a range of concentrations of potassium tellurite, from 10 to 0·02 mg/L. These were incubated at 37°C overnight and examined for the number and appearance of sorbitol-fermenting and NSF colonies.

Growth of strains on TC-SMAC medium

TC-SMAC medium was prepared by supplementing SMAC medium (Oxoid, CM813) with potassium tellurite 2·5 mg/L (from filter-sterilised aqueous potassium tellurite 3·5%) and cefixime 0·05 mg/L. Strains were grown for 18 h in nutrient broth at 37°C, diluted 1 in 10^6 in nutrient broth and then applied with a multipoint inocular to TC-SMAC and SMAC plates to give *c.* 10^2 organisms/spot inoculum. The plates were incubated for 18 h at 37°C and examined to determine whether growth on TC-SMAC was absent or markedly reduced with compared with growth on SMAC.

Comparison of CR-SMAC and TC-SMAC for isolating VT$^+$ E. coli O157 from bovine rectal swabs

Rectal swabs (391) were taken from cattle at an abattoir during an investigation of carriage of VT$^+$ *E. coli* O157. The swabs (Transwabs, Medical Wire Co.) were taken immediately after slaughter, transported to the laboratory on the same day and inoculated on to CR-SMAC medium[15] (SMAC supplemented with cefixime 0·05 mg/L and rhamnose 0·5%) and TC-SMAC medium. The plates were incubated at 37°C overnight. Non-fermenting colonies were screened with a latex test[20] for detecting *E. coli* O157 and latex-agglutinating colonies were confirmed as VT$^+$ *E. coli* O157 by agglutination to titre with *E. coli* O157 antiserum, standard biochemical tests, VT1 and VT2 gene probe tests and cell culture assay as described previously.[5]

Results

Tellurite MICs of strains

The potassium tellurite MICs of the strains tested are shown in table I. At a potassium tellurite concentration of 4 mg/L 56 of 70 non-O157 strains of *E. coli* and 11 of 38 VT$^+$ *E. coli* O157 strains from cattle were inhibited but all 38 strains of VT$^+$ *E. coli* O157 from human faeces grew.

Resolving mixed cultures

The incorporation of potassium tellurite 2·5 mg/L into SMAC gave the best inhibition of sorbitol-fermenting colonies while preserving colonial size and number of colonies of the NSF strain of VT$^+$ *E. coli* O157.

Effect of the addition of tellurite to SMAC on the growth of strains

The results are shown in table II. Of 397 strains of VT$^+$ *E. coli* O157, all except one grew on TC-SMAC without significant reduction in size or number of colonies whereas 67 of 100 strains of non-O157 *E. coli* from human faeces either failed to grow or showed significant reduction in growth on TC-SMAC when compared to growth on SMAC. All 22 strains of *S. sonnei* grew on TC-SMAC but with somewhat reduced colonial size. The growth of almost all of the strains of the other NSF species tested was largely or completely inhibited on TC-SMAC.

Comparison of CR-SMAC and TC-SMAC for isolating VT$^+$ E. coli O157 from bovine rectal swabs

When 391 bovine rectal swabs were cultured on TC-SMAC medium, 26 isolates of VT$^+$ *E. coli* O157 were detected after 206 NSF colonies had been screened. However, when the swabs were cultured on CR-SMAC medium, only nine isolates of VT$^+$ *E. coli* O157 were found after screening 47 NSF colonies. Eight of these nine strains had also been isolated on TC-SMAC. The difference in the number of isolates

Table I. MICs of potassium tellurite for bacterial strains in broth

Organism	Number tested	MIC 50 (mg/L)	MIC 90 (mg/L)
VT+ *E. coli* O157			
humans	38	32	250
cattle	38	8	125
Total	76	32	125
E. coli non-O157	70	2	16
Proteus spp.	20	500	> 500
Morganella morganii	10	125	250
Providencia spp.	10	⩽ 0·5	1
Aeromonas spp.	24	⩽ 0·5	2

Table II. Growth of bacterial strains on TC-SMAC compared with growth on SMAC

Organism	Number of strains tested	Number without marked inhibition of growth on TC-SMAC
Non-O157 *E. coli* from human faeces	100	33
VT+ *E. coli* O157 from human faeces	315	314
VT+ *E. coli* O157 from cattle faeces	82	82
Shigella sonnei	22	22
Proteus spp.	26	0
Morganella morganii	10	0
Providencia spp.	10	0
Aeromonas spp.	103	5
Plesiomonas spp.	8	0

between the two media was significant ($\chi^2 = 8{\cdot}28$, $p < 0{\cdot}01$).

Discussion

More sensitive methods for detecting VT+ *E. coli* O157 in man, animals, food and the environment are required. Current methods rely on differential sugar fermentation and do not select VT+ *E. coli* O157 from other strains of *E. coli*; therefore, they lack sensitivity. The new medium (TC-SMAC) gave substantial suppression of non-O157 strains and also inhibited such NSF bacteria as *Proteus* spp. by means of the cefixime, and *Providencia* spp. and *Aeromonas* spp. by means of the tellurite content. Rhamnose has been incorporated into CR-SMAC[15] because its fermentation differentiated many NSF, non-O157 strains of *E. coli* from O157 strains. We did not incorporate rhamnose in the new medium because it would increase the cost substantially and the six NSF, non-O157 strains of *E. coli* tested were all inhibited by low concentrations of tellurite, suggesting that addition of rhamnose may not be necessary.

The suppression of non-O157 *E. coli* on TC-SMAC uncovered a large number of NSF colonies (206 among the 391 specimens) that required screening by latex agglutination for O157. This is a considerable increase over the 47 colonies that required screening from CR-SMAC, albeit with a much increased yield in positive isolations from TC-SMAC. These non-O157 strains were not characterised further during the present study as they were of bovine origin and frequently cannot be identified by our standard range of biochemical tests. Characterisation could usefully be done in future to determine if other measures could suppress them or distinguish them from VT+ *E. coli* O157 and to determine if some of them carry genes for verocytotoxin production.

The mechanism of tellurite resistance in VT+ *E. coli* O157 is not known nor whether it is chromosomally or plasmid mediated. Tellurite resistance has been reviewed recently.[21] Intrinsic resistance in Enterobacteriaceae is often plasmid mediated and usually involves plasmids belonging to the HI, HII and P incompatibility groups. Chromosomally-mediated resistance can be induced in tellurite-sensitive strains of *E. coli*.[22] Possible mechanisms of resistance include reduced uptake via the phosphate transport pathway[22] and reduction to metallic tellurium.

Although the aim was to improve isolation methods for VT+ *E. coli* O157, it would be of interest to know whether verocytotoxigenic strains of other serotypes are also resistant to tellurite. Preliminary work on a small number of bovine strains of assorted serotypes suggests that some are resistant and some are not. However, most ferment sorbitol and TC-SMAC would not be useful for their isolation.

Tellurite is selective for VT+ *E. coli* O157 and markedly improved the rate of isolation from cattle rectal swabs. Further studies are needed with TC-SMAC to determine whether it will improve isolation rates from human faeces and from food specimens.

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