Research Article

Comparative Evaluation of Sorbitol MacConkey and Chromogenic Rainbow Agar for Detecting *Escherichia coli* O157:H7

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ABSTRACT

Enterohemorrhagic *Escherichia coli* (EHEC) and specifically serotype O157:H7 are a significant cause ofhemorrhagic gastrointestinal disease and the hemolytic uremic syndrome. The purpose of this investigation was to compare the Rainbow Agar with the conventional Sorbitol MacConkey Agar SMAC. Ninety four E. coli strains, including O157, O111 , O26 , O103 and O145 serogroups from stool samples were examined on Rainbow Agar O157. EHEC O157 could readily be isolated and recognized uniquely by typical black colonies. Some other EHEC also stand out as blue-black, whereas O26 and some other EHEC strains were purple , red or pink and indistinguishable from pathogenic strains of E. coli. SMAC had sensitivities of 75% and specificity of 13.33 % for the identification of all E.coli serotypes while Rainbow Agar (RA) had sensitivities of 100% and specificity of 95.74 %. RA was found to be more useful and accurate in defining different E.coli serotypes.

Keywords: *E. coli* O157:H7, Sorbitol MacConkey SMAC, Rainbow Agar (RA).

INTRODUCTION

Escherichia coli O157:H7 is an important food- borne pathogen and can cause diarrhoea, haemorrhagic colitis, and haemolytic uraemic syndrome HUS[1] through the production of the Shiga-like toxins Stx1 and Stx2 and other probable virulence factors[2, 3]. The agar medium most commonly used for the isolation of E. coli O157:H7 is Sorbitol–Mac Conkey agar(SMAC). These media contain sorbitol replacing the lactose of the standard MacConkey medium[4, 5]. Unlike other E. coli, isolates of serotype O157:H7 do not ferment D-sorbitol within 24 h, lack glucuronidase activity, and do not grow at 45.5 C so-called typical E. coli O157:H7 . Sorbitol-nonfermenting colonies, indicative of the typical E. coli O157:H7, are colorless on this medium.[6]

Recently, new selective media have been developed to increase the effectiveness of E. coli O157:H7isolation, including Rainbow agar(O157 RB; Biolog, Hayward, USA), Rainbow Agar O157 has both selective and chromogenic properties that make it particularly useful for isolating pathogenic E. coli strains. The medium contains chromogenic substrates that are specific for two E. coli associated enzymes: β galactosidase (a blue-black chromogenic substrate) and β glucuronidase (a red chromogenic substrate).

E.coli Strain O157:H7 is typically glucuronidase negative so it forms unique and distinctive black or gray colonies.Many other non-O157 toxigenic strains overproduce β galactosidase relative to β glucuronidase on this medium and consequently they are typically colored purple, violet or blue.[6, 7]

Bacterial Strains

All bacterial strains isolated from 94 diarrheal case, were purified and identified biochemically as Escherichia Coli bacteria, then all the E.coli isolates were serologically typed, using commercially available O Antisera (O157, O26, O103, O111, O145) (Denka Seiken, Japan).

MATERIALS AND METHODS

Sorbitol MacConkey SMAC (Liofilchem,Italy). The agar plates are clear and rose colored. Sorbitol fermentation and the pH indicator, neutral red, are used to detect sorbitol-positive colonies (red in colour). Sorbitol-negative strains (*E.coli* O157:H7) form colorless colonies.

Rainbow agar O157 (Biolog, USA): The agar plates are clear and colorless. On Rainbow agar O157, *E. coli* strains grow, yielding colonies ranging in color through various shades of red, magenta, purple, violet, blue, and black. The typical *E. coli* O157 strains form distinctive charcoal grey or black colonies. Other glucuronidase positive strains gave red or magenta coloured colonies.

Statistical analysis

For comparison of media, percentages for sensitivity and specificity were calculated, where appropriate, as follows:

Sensitivity % =True Positives ×100 /(True Positives+ False Negatives).

Specificity % =True Negatives×100 (True Negatives +False Positives).

RESULTS AND DISCUSSION

Results

Epidemiology

We found that from the 94 isolate of Escherichia coli The most common serogroups were O26 (40.42%), O111 (10.64%), O103 (10.64%), O145 (6.38%), O157 (4.25%), And other serotypes (27.66%)

Culture on SMAC

The results of the identification of *E. coli* serotypes on SMAC culture media are summarized in Tables 1, 2 ,3.

Culture on Rainbow Agar (RA)

The results of the identification of *E. coli* serotypes on RA culture media are summarized in Tables 4, 5.

Table 1: Relation between Serotypes and ferment sorbitol

Media	Serotype	Isolates (n)	Ferment sorbitol isolates (n)	Non Ferment sorbitol isolates (n)
SMAC	O26	38	3	35
SMAC	O103	10	2	8
SMAC	O111	10	-	10
SMAC	O145	6	-	6
SMAC	O157	4	1	3
SMAC	Other serotypes	26	7	19

Table 2: Serotypes of E.coli O157 and other than O157 on SMAC medium

Media	Serotype	Isolates (n)	Ferment sorbitol isolates (n)	Non Ferment sorbitol isolates (n)	Correct results (%)	False positive results (%)
SMAC	O157	4	1	3	75 %	-
SMAC	Other than O157	90	12	78	13.3 %	87 %

Table 3: sensitivity and speicificity of Sorbitol MacConkey (SMAC) in Ecoli O157 isolating

Media	False negative results	False positive results	True negative results	True positive results	Sensitivity %	Specificity %
SMAC	1	78	12	3	75	13.33

Table 4: E.coli serotype colors on Rainbow agar (RA)

White color	Purpule color	Violet color	Blue color	Black-gray color	Isolates (n)	Serotype	Medium
0	34	0	0	4	38	**O26**	**RA**
0	0	10	0	0	10	**O111**	**RA**
0	0	7	3	0	10	**O103**	**RA**
0	2	4	0	0	6	**O145**	**RA**
0	0	0	0	4	4	**O157**	**RA**
6	0	12	8	0	26	**Other serotypes**	**RA**

Table 5 : sensitivity and speicificity of Rainbow Agar (RA) in E.coli serotypes identification

Medium	True positive results	True negative results	False positive results	False negative results	Sensitivity %	Specificity %
RA	4	86	4	0	100	**95.74**

DISCUSSION

SMAC agar is the current standard for the detection of O157. While inexpensive and easy to use, but we found on our study this medium lacks sensitivity and Specificity to detect E.coli serotype O157.

In our study SMAC agar had a sensitivity and a specificity of 75% and 13.33% respectively. one strain out of the 4 strains of *E. coli* O157 tested (atypical *E. coli* O157:H7 strains) gave a false negative response, and don't ferment sorbitol. While Rainbow agar had a sensitivity and a specificity of 100% and 95.74%, Although this medium bears 0% false negative results. our results were similar to results in Bettelheim study[8], which has evaluated and compared RB agar with SMAC agar and showed that RB agar was far superior for the isolation of *E. coli* O157:H7 to the SMAC agar. In his study, 585 clinical isolates,

including *E. coli* O157, O111 and O113 serogroups, were examined on RB agar.

Some enterohaemorrhagic *E. coli* EHEC. other than seotype O157:H7 also stand out as blue-black colonies, whereas O113 and some other EHEC strains were mauve, red or pink and were indistinguishable from SLT-negative strains of *E. coli*. In our study, strains of *E. coli* O157:H7 could be readily isolated and recognized by their typical black colonies on RB agar.

CONCLUSION

The results of our study suggest that, when comparing SMAC culture and Rainbow agar for the detection of Escherichia coli O157 we found that Rainbow agar is better and more specific than SMAC for detection *E.coli* O157.

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