Comparison of five selective media for beta-haemolytic streptococci

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SUMMARY Five selective media for β -haemolytic streptococci were tested and compared with the conventional blood agar plate using 200 throat swabs from children with possible streptococcal pharyngitis. The medium described by Liebermeister and Braveny, which is based on the reduction of nutrients and enhancement of the haemolytic activity of β -streptococci, was markedly superior to the other selective media containing inhibiting agents.

The detection of group A streptococcal pharyngitis is of great importance because of the need to prevent its potential sequelae, rheumatic fever and rheumatic heart disease. A reliable diagnosis is based on identification of the aetiological agent.

The isolation of β -haemolytic streptococci group A from throat swabs may cause problems when there is a profuse growth of other micro-organisms and the streptococci are present in only small numbers. In recent years, several selective media for β -streptococci have been reported. Most investigators used antibiotics or chemical agents as selective inhibitors.¹⁻⁷ A selective medium based on the reduction of nutrients

and enhancement of the haemolytic activity of β -streptococci has also been described.⁸ From a variety of selective media, five were tested under the same conditions and compared with conventional sheep blood agar.

Material and methods

MEDIA

The different media were prepared according to the instructions of the authors.^{1 3 6-8} They are summarised in Table 1, which also lists the agar base, blood source, and inhibiting agents. Sheep blood agar (SBA) containing Columbia Agar Base (BBL) and 5% sheep blood was used as the control medium.

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Table 1 *Beta-haemolytic streptococcus: selective media evaluated*

Selective medium	Reference	Agar base (%)		Sheep blood (%)	Inhibiting agent (µg/ml)	
I	8	*Peptone from meat	0.1	7	—	
		Beef extract	0.06		—	
		Yeast extract	0.05		—	
		L + -lysine	0.002		—	
		Sodium chloride	0.6		—	
		di-Sodium hydrogen phosphate	0.2		—	
		Agar	1.5		—	
II	3	Trypticase Soy		6	Sulphamethoxazole	23.75
		Agar (Difco)	2.0		Trimethoprim	1.25
III	1	Trypticase Soy		5	Neomycin	30
		Agar (Difco)	2.0			
IV	7	Tryptose (Difco)	2.0	6	Neomycin	30
		Agar	1.5		Nalidixic acid	15
		Sodium chloride	0.5			
V	6	Heart Infusion		7	Sodium azide	100
		Agar (Difco)	4.0		Crystal violet	2
		Beer yeast extract	0.5			
		Sodium chloride	2.5			
		Urea	1.0			

*Ingredients of "β-Streptococcus Elective Agar Base according to Liebermeister and Braveny". E. Merck, Darmstadt.

THROAT SWABS

Two hundred throat swabs from children with possible streptococcal pharyngitis were placed in 2 ml peptone water and shaken vigorously; 0.1 ml of the suspension was spread uniformly on the surface of the selective media and on sheep blood agar. After incubation for 24 hours at 37°C in air the plates were examined for the presence of β -haemolytic colonies. Subcultures of the isolated colonies were made on sheep blood agar, and bacitracin disks were applied.

Results

Seventy-three (36.5%) of the 200 throat swabs cultured on the selective media and on the control medium were positive for β -haemolytic streptococci group A, 71 (35.5%) were recovered on medium I. Of the two strains that were missed by this medium, one grew only on SBA and one on medium II. The isolation rate on medium I was 5.5% higher than that obtained on SBA. The other selective media yielded a lower percentage of positive cultures compared with the SBA. Table 2 shows the semiquantitative colony counts of streptococci group A from the 73 positive throat swabs on the different selective media and on SBA. Medium I recovered the highest numbers of colonies.

Discussion

The purpose of this study was to test and compare five selective media for β -haemolytic streptococci under the same conditions. In most of these media (Table 1) the selectivity is based on partial inhibition of the normal throat flora by antibiotics (media II,

Table 2 Recovery of β -haemolytic streptococci group A from 200 throat swabs on different media including semiquantitative colony counts

Medium	No. of positive cultures	Colony counts of group A streptococci		
		1-20	21-100	> 100
I	71	10	19	42
II	45	6	9	30
III	37	7	11	19
IV	41	6	17	18
V	48	8	13	27
SBA	60	11	21	28

III, and IV) or chemical agents (medium V). Owing to the lack of nutrients in medium I the colony size of the grown bacteria, including β -haemolytic streptococci, is greatly reduced. On the other hand, the production of streptolysin S is promoted by the addition of yeast nucleic acid and lysine and the β -haemolytic streptococci are easily identified by their characteristic large haemolytic zone (Figure).

Medium I yielded the highest isolation rate of 35.5%, which was 5.5% higher than that obtained with the blood agar plate. All the other selective media yielded lower isolation rates than the blood agar plate. Some investigators^{1 6 7} reported an enhanced recovery rate of about 1% when selective inhibitory media are compared with blood agar plates and Braveny⁹ achieved an improvement of 11.6% with their medium based on reduction of nutrients. All these authors used a direct plating method. In this study the specimens were diluted in peptone water to provide a more consistent inoculum on the different media. As a result the quantity of all bacteria was diminished and the recovery rate of β -haemolytic streptococci was affected by the growth

Culture of throat swab on medium I (left) and sheep blood agar (right). On medium I the β -haemolytic streptococcal colonies are easily identified by the haemolytic zone.

rate on the specific medium. Growth on media with inhibiting agents is lower than on a nutrient agar. This explains the lower recovery rates on the selective media containing inhibiting agents in this study.

On medium I the recovery of β -haemolytic streptococci is not only improved, compared with the other selective media and the blood agar plate, but also has some other advantages. The characteristic growth of β -haemolytic streptococci—very small colonies surrounded by a large haemolytic zone—makes it easy to distinguish them from other haemolytic colonies which exhibit only a small and incomplete haemolytic zone. Thus a prompt and reliable diagnosis can be made without microscopy and subculture, which is not always possible when other selective media are used. The normal flora is not completely inhibited on medium I, and an evaluation of the quantitative relationship between β -haemolytic streptococci and this flora is therefore possible. An additional practical advantage of medium I over media containing antibiotics is that it can be stored for a longer time. Because of these advantages we recommend the medium of Liebermeister and Braveny⁹ for routine testing in clinical laboratories.

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