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Use of colistin and sorbitol for better isolation of *Serratia marcescens* in clinical samples

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SUMMARY

A comparison was made of different culture media and procedures for detection of *Serratia marcescens* from faecal, pharyngeal and ocular swabs collected from 213 neonates. MacConkey agar and MacConkey agar with sorbitol (1%) and/or colistin (200 i.u./ml) were used both for primary isolation and after enrichment using Mossel Enterobacteriaceae broth with colistin (200 i.u./ml). The use of MacConkey agar supplemented with colistin for primary isolation improved considerably the isolation rate of *S. marcescens* from faecal swabs but not from pharyngeal swabs; the number of ocular isolations were insufficient to demonstrate differences between procedures. Moreover the enrichment procedures consistently increased the number of *S. marcescens* isolates especially from pharyngeal and ocular swabs. Use of sorbitol made detection of *S. marcescens* from clinical specimens easier and time- and cost-efficient.

INTRODUCTION

Serratia marcescens is considered a major agent of nosocomial infection all over the world and several *S. marcescens* outbreaks which were difficult to control have been described. The source of contamination, though sometimes unidentified, have been associated with a wide range of patient care devices. The isolation of *S. marcescens* has been reported from all types of clinical specimens and problems may arise with some strains which are multiply antibiotic resistant. Thus the ability of a laboratory to effect prompt isolation and identification of *S. marcescens* strains has become increasingly important.

During recent years many papers have reported the results of studies on primary selective media (Schreier, 1969; Slotnick & Dougherty, 1972; Farmer, Silva & Williams, 1973; Traub & Kleber, 1975; Starr *et al.* 1976; Lovell & Bibel, 1977), on procedures for isolation from water samples (Dalbke & Lymch, 1984) and raw sewage (Lynch & Kenealy, 1976), and on enrichment factors (Burger & Bennett, 1985).

In this paper we report the results of a comparison of very simple direct and

enrichment procedures utilizing MacConkey Agar as the plating medium base, sorbitol as a discriminating medium component, and colistin as a selective agent both for plating media and broth enrichment.

MATERIALS AND METHODS

Media. The following media have been used:

(1) MacConkey agar no. 3 (Oxoid Limited, Basingstoke, Hampshire, England);

(2) MacConkey Colistin agar, consisting of MacConkey agar no. 3 with 200 i.u./ml colistin (UCB SpA, Turin, Italy) added aseptically after autoclaving the base medium;

(3) MacConkey Colistin Sorbitol agar, consisting of MacConkey agar no. 3 with 200 i.u./ml colistin and 0·01 g/ml sorbitol (Fluka, Buchs, Switzerland) added aseptically after autoclaving the base medium;

(4) Mossel Enterobacteriaceae Enrichment Broth (Merck. Darmstadt. Federal Republic of Germany), supplemented with 200 i.u./ml colistin.

Addition of colistin to the culture media, both MacConkey agar and Mossel broth, consistently inhibits the growth of almost all Enterobacteriaceae and non-fermentative Gram-negative rods. Only *Proteus* spp., *S. marcescens* and, rarely, a few strains of *Pseudomonas* spp. other than *P. aeruginosa* are able to grow on these media (Neuman, 1979).

Both on MacConkey agar (MA) and MacConkey Colistin agar (MCA) *S. marcescens* forms colonies indistinguishable from other lactose-negative and non-fermentative Gram-negative bacteria. However, on MacConkey Colistin Sorbitol agar (MCSA) *S. marcescens* forms typical reddish colonies clearly distinguishable from those produced by the other bacteria growing on this medium.

Clinical isolates. Over a 7-month period 213 neonates admitted to the nursery at the University Hospital of the second School of Medicine in Naples were cultured for *S. marcescens* on the third day after birth. From each infant, faecal, pharyngeal and ocular swabs were obtained, were transferred into Amies Transport Medium (Oxoid Limited, Basingstoke, Hampshire, England) and sent to the laboratory.

The specimens were processed as follows: each swab was streaked onto plates of MA, MCA and MCSA respectively, and plates were incubated at 37 °C overnight. After that each swab was transferred into Mossel Enterobacteriaceae Enrichment Broth supplemented with colistin. Then the broth was incubated at 37 °C overnight and after incubation subcultures were performed on MA, MCA and MCSA. The plates were incubated at 37 °C overnight.

The *S. marcescens*-like isolates (lactose negative colonies on MA and MCA, and sorbitol positive on MCSA) were confirmed by routine biochemical procedures (Kelly, Brenner & Farmer III, 1985). The species identification was carried out as described by Grimont & Grimont (1984).

Statistical methods. The statistical analysis of the data was carried out using the Cochran's Q test for the comparison of matched samples (Cochran, 1950; Fleiss, 1965). Whenever needed McNemar's test was performed using the continuity correction for nominal scale data.

RESULTS

Table 1 shows the recovery rates of *S. marcescens* from faecal, pharyngeal and ocular swabs respectively, with and without using the enrichment procedure, on different culture media. It is quite evident that with faecal swabs there is a clear improvement in the number of isolates using MA supplemented with colistin, both with and without sorbitol, compared with MA, when no enrichment procedure is carried out. Indeed, the Q statistics ($Q = 24{\cdot}82$; 1 degree of freedom (D.F.), $P < 0{\cdot}001$) confirm a significant difference between MA and the culture media with colistin (MCA and MCSA). Conversely the MA seems to be more effective, in primary isolation, than the other media ($Q = 11{\cdot}31$; 1 D.F., $P < 0{\cdot}001$) in detecting *S. marcescens* from pharyngeal swabs, with 76 positives from 213 specimens (35·7%), whereas MCA and MCSA showed isolation rates of 30·5 and 31·0% respectively. There were too few ocular isolations by direct plating to demonstrate significant differences between media with and without colistin. The enrichment procedure was much more effective than the direct culture in recovering *S. marcescens* from pharyngeal ($Q = 190{\cdot}2$; 1 D.F., $P \ll 0{\cdot}001$) and ocular ($Q = 54{\cdot}2$; 1 D.F., $P \ll 0{\cdot}001$) swabs, whereas for the faecal swabs a clear improvement over the direct culture procedure was observed only when MA was used for primary isolation of *S. marcescens* (McNemar $\chi_c^2 = 15{\cdot}56$; $P < 0{\cdot}001$); indeed the recovery rate was 32·9% using MA only, and rose to 44·1% using MA after enrichment.

No significant differences, in all the three groups of clinical samples, were found among the recovery rates of MA, MCA and MCSA, when these culture media were used for subculturing from the enrichment broth.

Supplementation of MCA with sorbitol clearly did not improve the effectiveness of the medium in recovering *S. marcescens*, the percentages of isolations using MCA being about the same as those obtained using MCSA, both with and without enrichment. Nevertheless the presence of sorbitol in the culture medium consistently reduced the number of *S. marcescens*-like colonies which underwent routine identification procedures, as shown in Table 2. The major point is that almost all the *S. marcescens*-like colonies grown on MCSA were confirmed as *S. marcescens*, with agreement ranging between 91 and 100%. The lowest ratio of confirmed to suspected colonies was observed using MA, and only a slight improvement resulted from using MCA.

DISCUSSION

During the last 30 years laboratory management of clinical samples for infectious disease diagnosis has been complicated by several factors such as the increasing number of new opportunistic agents and the need to adopt more sophisticated microbiological techniques. In hospital infection control, particularly during outbreaks, there is a need for rapid identification of all the carriers of the causal agent. The use of a large variety of primary isolation media during an epidemic is time consuming and expensive, and in some instances can overload the laboratory.

The rationale of our study was to test the effectiveness of MCA and MCSA in optimizing and making easier the detection of *S. marcescens* strains.

Table 1. *Results of* Serratia marcescens *isolations by different procedures*

Specimen	Total no.	No. positive (%)*	No. positive (%) in direct culture			No. positive (%) after enrichment		
			MA	MCA	MCSA	MA	MCA	MCSA
Faecal	213	109 (51·2)	70 (32·9)	93 (43·7)	91 (42·7)	94 (44·1)	95 (44·6)	98 (46·0)
Pharyngeal	213	116 (54·5)	76 (35·7)	65 (30·5)	66 (31·0)	111 (52·1)	113 (53·1)	114 (53·5)
Eye fluid	213	15 (7·0)	2 (0·9)	2 (0·9)	4 (1·9)	13 (6·1)	15 (7·0)	15 (7·0)

* With any procedure.
MA, MacConkey agar; MCA, MacConkey Colistin agar; MCSA, MacConkey Colistin Sorbitol agar.

Table 2. *Number and percentage of* Serratia marcescens *strains of the total of suspected colonies isolated*

Medium	Faecal swabs		Pharyngeal swabs		Eye secretion	
	No. susp.*	No. (%) *Serratia marcescens*	No. susp.*	No. (%) *Serratia marcescens*	No. susp.*	No. (%) *Serratia marcescens*
MA	154	70 (45·5)	110	76 (69·1)	18	2 (11·1)
MCA	141	93 (66·0)	68	65 (95·6)	9	2 (22·2)
MCSA	100	91 (91·0)	66	66 (100·0)	4	4 (100·0)
E-MA	166	94 (56·6)	130	111 (85·4)	27	13 (48·1)
E-MCA	162	95 (58·6)	119	113 (95·0)	20	15 (75·0)
E-MCSA	98	98 (100·0)	114	114 (100·0)	15	15 (100·0)

* Suspected colonies.
MA, MacConkey agar; MCA, MacConkey Colistin agar; MCSA, MacConkey Colistin Sorbitol agar; E, enrichment on Mossel Enterobacteriaceae Colistin broth.

Our first experience with the use of colistin for this purpose started in 1982. At that time we used MA supplemented with 200 i.u./ml colistin; in that previous study we investigated the circulation of *S. marcescens* strains during an outbreak in a neonatal intensive care unit (Montanaro *et al.* 1984). The choice of this antibiotic as a selective agent depended upon the fact that resistance to colistin is very frequent in the genus *Serratia* and almost constant in *S. marcescens* (Grimont & Grimont, 1984).

Our findings confirm that the use of colistin as a selective agent is almost always advantageous. Enrichment followed by subculture on MCSA is considered the best procedure particularly to process pharyngeal and ocular swabs. It must be emphasized that with faecal specimens none of the procedures approach the detection of the maximum number of positive results (109 isolations). In accordance with our results, we suggest using both primary isolation and enrichment procedures to reduce the number of false negatives, stressing that primary isolation must carried out on a medium containing colistin (MCA or MCSA).

The decision to use sorbitol was closely related to the use of colistin as the selective agent. While colistin strongly inhibits the growth of several Enterobacteriaceae on MacConkey Agar, it may lead to an overgrowth of *Proteus* spp. and of rare Gram-negative non-fermenters. As sorbitol is fermented by all *S. marcescens* strains but not by *Proteus* spp. or non-fermenters (Kelly, Brenner & Farmer III, 1985), its addition facilitates the differentiation of *S. marcescens*. In our experience this is clearly demonstrated by the reduction in number of *S. marcescens*-like colonies. The use of MacConkey Colistin Sorbitol agar is particularly useful in samples with heavy mixed flora. Where this is less of a problem, as with ocular swabs, the use of a medium supplemented with sorbitol does not increase the isolation rate.

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