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A New Plating Medium for the Isolation of Enteric Pathogens

II. Comparison of Hektoen Enteric Agar with S S and E M B Agar

SYLVIA KING AND WILLIAM I. METZGER

Department of Microbiology, Cook County Hospital, and The Hektoen Institute for Medical Research, Chicago, Illinois 60612

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During this study, 2,855 stool specimens from patients at Cook County Hospital were cultured for enteric pathogens. Hektoen Enteric Agar (HE) was compared with E M B and S S Agars by replicate samplings with both direct and indirect methods. *Shigella* species were recovered more than twice as often on HE Agar as on S S Agar by both methods. With the direct method only, out of 98 *Shigella* isolated, 97 were isolated from HE Agar, 74 were recovered from E M B Agar, and 40 were found on S S Agar. In addition, HE yielded better isolation of *Salmonella* strains than did S S or E M B by either direct or indirect methods. The greater efficiency of HE medium is discussed with respect to colonial recognition of enteric pathogens.

Many media have been devised over the years for the isolation of enteric pathogens, some having been designed especially for the isolation of specific organisms, whereas others are broader in spectrum. Unfortunately, the latter type of media are also very supportive for the numerous gram-negative organisms normally present in the gastrointestinal tract. More recently, complex plating media have been developed that are not only differential but also highly selective. The two most commonly used in clinical laboratories are S S Agar and Desoxycholate Citrate Agar; these agars are very similar in their selectivity patterns.

In the present study, we have compared Hektoen Enteric Agar (HE) with the two media currently in use in our diagnostic laboratory, S S and E M B Agars.

MATERIALS AND METHODS

The material used in this study was obtained from stool specimens from the wards of Cook County Hospital. These were specimens submitted for routine diagnostic purposes, and the periods of study were November 1966 through March 1967 (1) and September through November 1967 (2).

The stools or the material on rectal swabs was emulsified in a small amount of sterile normal saline. The emulsion was inoculated uniformly on E M B, S S, and HE Agar plates by means of a cotton swab. For isolation of colonies, each plate was streaked with a sterile wire loop. The remainder of the specimen in saline was added to a tube of Selenite-F Enrichment broth. The following day, approximately 18 to 20 hr later, growth in the broth was subcultured by means of a sterile swab onto S S and HE plates.

All suspicious organisms from all plates were subcultured onto Triple Sugar Iron Agar. Those organisms which could not be eliminated from among these slants were transferred to semisolid mannitol and sucrose and to tryptone broth. Other biochemical media employed, when indicated, were lactose, salicin, urea, malonate, and lysine decarboxylase (1). All organisms identified as *Salmonella* or *Shigella* were typed serologically by use of both polyvalent and type specific antisera. Positive salmonellae were sent to the regional Illinois State Health Laboratory for species identification.

RESULTS AND DISCUSSION

During this study, no enteric pathogens other than *Salmonella* or *Shigella* were isolated. HE medium was far superior to S S Agar for the isolation of *Shigella* species (Table 1). Out of a total of 98 shigellae isolated from all media, 97 were recovered on HE and only 40 on S S Agar. The recovery of *Salmonella* species also was better on HE Agar than on S S Agar, but the differential was less striking.

The numbers of *Salmonella* and *Shigella* species isolated on each medium by both direct plating of specimens and indirect plating from Selenite-F broth are shown in Table 2. The largest numbers of shigellae were isolated by direct plating on E M B and HE Agars. We did not recover any shigellae from S S Agar alone, and we only recovered 1 from E M B Agar alone and 14 from

HE Agar alone. Plating from Selenite-F Enrichment broth resulted in the recovery of 61 shigellae; of these, 41 were isolated from HE Agar alone.

Although the recovery of salmonellae on HE Agar, as compared with S S Agar, was less dramatic than recovery of shigellae, it is obvious from Tables 1 and 2 that HE Agar was superior for for the isolation of this genus. The reason for the better recovery of shigellae than of salmonellae on E M B Agar is not immediately apparent.

The *Shigella* isolates were about equally divided between the B and D serological groups plus one from the C group. In all, 25 different species of *Salmonella* were isolated, the most common being *S. typhimurium*.

To confirm further the foregoing results, an additional 1,260 stool specimens were cultured directly on duplicate plates of S S and HE Agars as a corollary to routine cultures carried out in our diagonstic laboratory. The results (Table 3) show once more the better recovery of *Shigella* organisms on HE Agar as compared with S S Agar. Unfortunately, during this study period, the incidence of *Salmonella* isolations was generally low. However, the data demonstrate more frequent isolations of *Salmonella* on HE Agar than on S S Agar.

Table 4 shows the number of pathogens isolated on one or both duplicate plates of S S and HE Agars. Of the 10 *Salmonella* species recovered on HE Agar, 6 were isolated on both duplicate plates and 4 were isolated on one of the two plates. Of the 87 *Shigella* species recovered on HE Agar, 56 were recovered on both duplicate plates and 31 on one of the two plates. The same types of data are extractable from the table for S S Agar. The total number recovered on S S was 51, of which 28 were recovered on both duplicate plates and and 23 were recovered on one of the two plates. It is obvious that primary duplicate plating for the recovery of enteric pathogens is preferable to single plating. If a single plate had been used in this study, it is possible that 31 of 87 *Shigella* and 4 of 10 *Salmonella* might have been missed on HE Agar, and 23 of 51 *Shigella* and 5 of 7 *Salmonella* might have been missed on S S Agar. Although the advantages of multiple plating are obvious, the time and materials consumed in this process are deterrants.

The isolation of a pathogen is the primary function of diagnostic work; nevertheless, conservation of labor and materials becomes increasingly more important as the number of cultures in the diagnostic laboratory increases. It is important, therefore, to have a plating medium that facilitates identification of a given organism with a minimum of further investigation. One of the

TABLE 1. *Recovery of enteric pathogens from 1,595 stool specimens*

	Shigella		Salmonella	
	No.	Per cent	No.	Per cent
Direct plating				
Total isolations: all media	98		99	
Total isolations: one medium				
E M B	74	75.5	53	53.5
S S	40	41	90	91
HE	97	99	96	98
Indirect plating from Selenite				
Total isolations: both media	61		138	
Total isolations: one medium				
S S	20	33	118	85.5
HE	60	98	135	99

TABLE 2. *Patterns of Shigella and Salmonella recovery on different plating media*

Medium	Direct plating				Indirect plating from Selenite			
	Salmonella		*Shigella*		*Salmonella*		*Shigella*	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
E M B alone	1	1.0	1	1.0				
S S alone	1	1.0	0	0	3	2.2	1	1.6
HE alone	7	7.1	14	14.3	20	14.5	41	67.2
E M B, S S, and HE	50	50.5	30	30.6				
E M B and S S	1	1.0	0	0				
S S and HE	38	38.4	10	10.2	115	83.3	19	31.2
E M B and HE	1	1.0	43	43.9				
Total	99		98		138		61	

TABLE 3. *Recovery of enteric pathogens from 1,260 stool specimens by use of paired plates of S S and HE Agars*

Organism	Total no. of recoveries	Recoveries on S S Agar	Recoveries on HE Agar
Shigella	90	46 (51.1%)	85 (94.4%)
Salmonella	14	7 (50%)	10 (71.4%)

TABLE 4. *Patterns of Shigella and Salmonella recovery on paired plates of S S and HE Agars*

Medium		*Salmonella*		*Shigella*	
S S	HE	No.	Per cent	No.	Per cent
−−[a]	−−	3[b]	21.4	2[b]	2.2
+−	−−	1	7.1	1	1.1
++	−−	0	0	0	0
++	+−	0	0	2	2.2
+−	−+	2	14.3	12	13.3
−−	−+	2	14.3	17	18.9
−−	++	2	14.3	20	22.2
−+	++	2	14.3	10	11.1
++	++	2	14.3	26	29.0
Total		14		90	

[a] Minus indicates that organisms were not isolated on the plate; plus indicates that organisms were isolated from plate.

[b] Three *Salmonella* species and two *Shigella* species were isolated only on E M B agar in the routine laboratory cultures.

ways to determine the efficiency of a medium is to compare ratios between the total number of positive subcultures to the total number of subcultures done (2). Table 5 shows that HE Agar is more than twice as efficient as S S Agar for the selection of *Shigella* colonies and slightly more efficient for the selection of *Salmonella* colonies. Obviously, the aptitude of the technician will have some bearing on data such as these.

TABLE 5. *Ratio of positive subcultures to total number of subcultures made from each of three plating media*

Medium	Total no. of subcultures	Positive *Salmonella* subcultures		Positive *Shigella* subcultures	
		No.	Per cent	No.	Per cent
E M B...	1,899	83	4.3	280	15
S S.....	4,706	246	5.2	363	7.7
HE.....	4,239	269	6.3	711	16.8

Comparative studies have demonstrated that HE Agar is significantly better than S S Agar for the isolation of *Shigella* species from stool specimens. Furthermore, HE Agar requires less time and materials than S S Agar for positive findings. HE Agar also appears to be somewhat more efficient than S S Agar for the isolation of *Salmonella*. It is suggested that HE Agar be substituted for S S Agar for stool culture work in the diagnostic laboratory.

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