APPLIED MICROBIOLOGY, Mar. 1970, p. 434-437
Vol. 19, No. 3

Printed in U.S.A.

Comparison of Media for Direct Isolation and Transport of Shigellae from Fecal Specimens

GEORGE K. MORRIS, JUDITH A. KOEHLER, EUGENE J. GANGAROSA, AND ROBERT G. SHARRAR
Epidemiology Program, National Communicable Disease Center, Atlanta, Georgia 30333

Received for publication 5 December 1969

Xylose-lysine-deoxycholate (XLD) agar, SS agar, and MacConkey agar for isolating shigellae from fecal specimens were compared. XLD agar was superior to both SS agar and MacConkey agar for isolating *Shigella sonnei*, and both XLD and SS agar were superior to MacConkey agar for isolating *S. flexneri*. Direct plating of the fecal specimens in the field resulted in a greater yield of shigellae as compared to transporting specimens to the laboratory either in holding media or enrichment broth. Buffered glycerol saline was superior to other transport media evaluated, yielding 83% of shigella isolates when plated within 48 hr as compared to direct plating. The combination of XLD agar and SS agar is recommended for direct isolation of shigellae, and, whenever possible, these solid media should be taken to the bedside and inoculated directly.

Many media have been recommended for the transport, enrichment, and isolation of shigellae. We evaluated three commonly used plating media for direct isolation and five transport or enrichment media to determine the optimal method for recovery of shigellae from fecal specimens.

MATERIALS AND METHODS

The clinical material for this study consisted of 4,228 fecal specimens, mostly in the form of rectal swabs, collected in the 5-year period 1965 to 1969. Of these 4,228 fecal specimens, 2,068 were plated on xylose-lysine-deoxycholate (XLD) and SS agars, and the remaining 2,160 were plated on XLD, SS, and MacConkey agars. Samples were collected as part of surveys for shigellosis in mental hospitals, in monkey colonies at the National Communicable Disease Center, and during epidemiologic investigations of outbreaks of acute shigellosis around the United States. A rectal swab was taken by gently inserting it beyond the rectal sphincter muscle. In the few cases that fresh stools were received, the swab was inserted into the stool, and the media were inoculated as with rectal swabs. Plating media were seeded immediately by rotating the swab on an area of the agar surface approximately 1 inch in diameter. The swab was rotated after each inoculation so that an unused side of the swab was exposed to each plate. The sequence in which the various media were plated with the swab was rotated so that all solid media were equally represented in being plated first, second, etc. Also, in some cases, multiple swabs were taken to inoculate transport or enrichment media. The following transport and enrichment media were utilized during the course of this study: buffered glycerol saline (3), Cary-Blair medium (2), silica gel (Protek-Sorb, Poly foil bag, Grace Davison Chemical, Baltimore, Md.), Gram-negative (GN) broth (5), and specimen preservative (SP) broth (4). After specimens in transport and enrichment media were received in the laboratory, they were plated on media similar to that used in the field for direct plating. Swabs in silica gel were rehydrated in Brain Heart Infusion broth for 2 hr at 37 C prior to inoculating the plating media. After inoculation, plates were streaked with a sterile loop and were incubated at 37 C for 20 to 24 hr.

After incubation, all colonies suspected of being shigellae were picked and inoculated into Triple Sugar Iron agar (Difco; TSI). All cultures showing TSI reactions suspicious of shigellae were typed serologically. These cultures, along with those negative serologically, were examined with the following biochemical tests: indole; methyl red; Voges-Proskauer; lysine, arginine, and ornithine decarboxylases (Moeller method); Christensen's citrate agar; Christensen's urea agar; motility; lactose, mannitol, and glucose fermentation broths (3); and acetate (9). Confirmation of selected isolates and the serological subgrouping were performed by the Enteric Bacteriology Laboratories, National Communicable Disease Center.

XLD agar was made from basic ingredients (8) in the early phases of the study, but, when commercial medium became available, the XL agar base (BBL) was used and heat-labile ingredients were added after autoclaving. SS agar (Difco) and MacConkey agar (Difco) were prepared from commercial dehydrated preparations.

RESULTS

Of the 4,228 fecal specimens studied, 2,068 specimens were plated on XLD and SS agars. Shigellae were isolated from 230 of these speci-

TABLE 1. *Recovery of shigellae on XLD and SS agars from fecal specimens*

Media combination	Method of plating		
	Direct	Indirect[a]	Total
XLD + SS	71	15	86
XLD alone	54	32	86
SS alone	44	14	58
Total on XLD	125	47	172
Total on SS	115	29	144
Total on both media	169	61	230
Total specimens examined	986	1,082	2,068

[a] Specimens received in transport media or enrichment broth.

TABLE 2. *Distribution of shigellae from 2,068 fecal specimens on two plating media (XLD and SS agars)*

Media	Species					
	S. dysenteriae[a]		*S. flexneri*		*S. sonnei*	
	No.	Per cent	No.	Per cent	No.	Per cent
XLD alone	2	66.7	46	31.5	38	46.9
SS alone	0	0	49	33.6	9	11.1
Both SS and XLD	1	33.3	51	34.9	34	42.0

[a] *S. dysenteriae* 2.

TABLE 3. *Recovery of shigellae on XLD, SS, and MacConkey agars from fecal specimens*

Media combination	Method of plating		
	Direct	Indirect	Total
XLD + SS + MacConkey	31	3	34
SS + MacConkey	3	0	3
XLD + MacConkey	6	1	7
SS + XLD	34	3	37
MacConkey alone	6	2	8
SS alone	23	0	23
XLD alone	22	7	29
Total on MacConkey	46	6	52
Total on SS	91	6	97
Total on XLD	93	14	107
Total all media	125	16	141
Total specimens examined	1,563	597	2,160

mens (Table 1). XLD agar appeared to be slightly superior to SS agar when the specimens were plated directly; 54 isolates of *Shigella* were obtained on XLD alone, whereas only 44 were isolated on SS alone. However, a distinct superiority of XLD over SS was noted when the two plating media were inoculated indirectly, i.e., after being held in transport or enrichment media. In the latter case, 32 specimens were positive on XLD alone and only 14 were positive on SS alone.

TABLE 4. *Distribution of shigellae on three plating media (XLD, SS, and MacConkey agars)*

Media	*Shigella* species			
	S. flexneri		*S. sonnei*	
	No.	Per cent	No.	Per cent
MacConkey alone	4	4.3	4	8.2
SS alone	21	22.8	2	4.1
XLD alone	15	16.3	14	28.6
SS and XLD	23	25.0	14	28.6
XLD and MacConkey	2	2.2	5	10.2
SS and MacConkey	3	3.3	0	0
XLD, SS, and MacConkey	24	26.1	10	20.4
Total isolations with XLD	64	69.6	43	87.8
Total isolations with SS	71	77.2	26	53.1
Total isolations with MacConkey	33	35.9	19	38.8

The distribution of the *Shigella* species on the two plating media was determined (Table 2). In the case of *Shigella flexneri*, the two media were approximately equal, but XLD agar was far superior to SS agar for isolating *S. sonnei* (38 isolates on XLD alone, but only 9 isolates on SS alone). Isolations of *S. dysenteriae* were too few to evaluate.

The 2,160 fecal specimens plated on XLD, SS, and MacConkey agars yielded 141 shigellae isolates (Table 3). When samples were plated directly, XLD and SS were individually superior to MacConkey (93, 91, and 46 isolates, respectively), but there was little difference between XLD and SS. With those samples, plated indirectly, with holding or enrichment media, XLD was superior to both SS and MacConkey agars (14, 6, and 6 isolates, respectively), but there was no difference between SS and MacConkey.

The species distribution (Table 4) indicated that, for the isolation of *S. flexneri*, XLD and SS agars are superior to MacConkey agar, but there was no apparent difference between XLD and SS agars. For isolation of *S. sonnei*, XLD was superior to both SS and MacConkey agars, but there was no apparent difference between SS and MacConkey agars.

Some specimens were inoculated into holding and enrichment media, and the analysis of those

TABLE 5. *Efficiency of various media used to transport fecal specimens for the isolation of shigellae*

Transport or enrichment media	Days media held before plating	No. fecal specimens examined	No. *Shigella* isolated by direct plating of fecal specimen	No. *Shigella* isolated when specimens plated from transport media	Per cent positive compared to direct plating
Buffered glycerol saline	1–2	36	18	15	83.0
	3–6	271	50	24	48.0
	7–10	203	45	17	37.8
Cary-Blair	1–2	298	68	18	27.5
	3–6	148	35	8	22.8
	7–10	295	27	8	29.6
GN broth	1–2	298	66	8	12.1
	3–6	125	28	2	7.1
	7–10	24	8	0	0
Silica gel	3	18	8	3	38.0
SP broth	7–10	148	14	1	7.1

samples for which results of direct plating were also available are shown (Table 5). Buffered glycerol saline was superior to either Cary-Blair medium or GN broth at each time period, and GN broth was the poorest when compared to the results of direct plating. The efficiency of both buffered glycerol saline and GN broth decreases with time. The efficiency of the Cary-Blair medium was intermediate to buffered glycerol saline and GN broth, and the effectiveness of Cary-Blair medium did not deteriorate with time in this study. The isolation of shigellae from 18 human rectal swabs by plating directly to XLD and SS was compared to plating indirectly after transport at room temperature in silica gel for 3 days. Eight shigellae were isolated by the direct method, whereas only three were isolated by the indirect method. Of 148 human fecal specimens, 14 yielded shigellae when plated directly, but only one of these yielded shigellae when held in SP broth for 7 to 10 days prior to plating.

DISCUSSION

The efficiencies of XLD and SS agars were similar when the fecal specimens were plated directly, but XLD agar was superior when the specimens were plated indirectly with transport or enrichment media. The efficiency of MacConkey agar was similar whether plated directly or indirectly. However, there were fewer isolates from Mac Conkey agar than from either XLD or SS agars when the specimens were plated directly. On indirect plating, the efficiency of MacConkey agar was comparable to SS agar and inferior to XLD agar. Neither MacConkey nor XLD was noted to show any advantage for the isolation of either *S. flexneri* or *S. sonnei*. SS agar, however, was more effective for isolating *S. flexneri* than *S. sonnei*. The low number of *S. sonnei* isolated on SS agar may be influenced by the large number of specimens of carrier-state individuals cultured in the monkey colony and mental hospitals in this study. Although *S. sonnei* I is isolated more frequently from acute cases, *S. sonnei* II is more commonly isolated from carriers (1). SS agar has been shown to be inhibitory for *S. sonnei* II but not for *S. sonnei* I (10).

The transport and enrichment media yielded shigellae less frequently than fresh fecal specimens inoculated directly on solid media. Buffered glycerol saline was the best of the five media evaluated, whereas GN broth, designed as an enrichment medium rather than holding medium, was the poorest. The recovery of shigellae from buffered glycerol saline and GN broth progressively decreased with time, after the rectal swabs were taken. Cary-Blair medium was intermediate to buffered glycerol saline and GN broth for isolating shigellae, and its effectiveness in maintaining shigellae did not decrease with time.

At least two solid media are recommended for direct isolation because lot-to-lot variation of individual commercial media has been reported (6, 7).

ACKNOWLEDGMENTS

We thank E. R. Eichner, R. W. Armstrong, and W. E. Woodward, who assisted in the collection of specimens while serving in the Epidemic Intelligence Service; Pamela Terry, Carolyn G. Dunn, and William T. Martin, for laboratory assistance during certain portions of this study; and W. H. Ewing and W. J. Martin for confirmation of isolates and for reviewing the manuscript.

LITERATURE CITED

1. Branham, S. E., S. A. Carlin, and D. B. Riggs. 1952. A comparison of the incidence of phases I and II of *Shigella sonnei* in cultures from acute infections and carriers. Amer. J. Public Health 42:1409–1413.
2. Cary, S. G., and E. B. Blair. 1964. New transport medium for shipment of clinical specimens. I. Fecal specimens. J. Bacteriol. 88:96–98.
3. Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis.
4. Hajna, M. S. 1955. A new specimen preservative for Gram-negative organisms of the intestinal group. Public Health Lab. Bull. Conf. State Prov. Public Health Lab. Dir. 13:59–62.
5. Hajna, M. S. 1955. A new enrichment broth medium for Gram-negative organisms of the intestinal group. Public Health Lab. Bull. Conf. State Prov. Public Health Lab. Dir. 13:83–89.

6. Morris, G. K. 1968. Evaluation of certain recommended techniques in detecting salmonellae. Salmonella Surveillance Report no. 74. National Communicable Disease Center, Atlanta, Ga.
7. Read, R. B., and A. L. Reyes. 1968. Variation in plating efficiency of salmonellae on eight lots of Brilliant Green Agar. Appl. Microbiol. 16:746–748.
8. Taylor, W. I. 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Amer. J. Clin. Pathol. 44:471–475.
9. Trabulsi, L. R., and W. H. Ewing. 1962. Sodium acetate medium for the differentiation of *Shigella* and *Escherichia* cultures. Public Health Lab. Bull. Conf. State Prov. Public Health Lab. Dir. 20:137–140.
10. Wheeler, K. M., and F. L. Mickle. 1945. Antigens of *Shigella sonnei*. J. Immunol. 51:257–267.