Clinical Evaluation of Enteric Media in the Primary Isolation of Salmonella and Shigella

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Received for publication 12 September 1973

Over a 1-year period, media for the isolation of enteric pathogens were compared on 455 stool specimens. Fifty-three pathogens were isolated, of which 56% were Shigella sonnei and 13% were Sh. flexneri. Of these isolates, 90% were found on xylose-lysine-desoxycholate agar, 87% on Hekton enteric agar, and 80% on MacConkey without crystal violet with 2% agar and 0.007% neutral red, but only 28% were recovered on Salmonella-Shigella agar. Less than one-half of the shigellae were recovered after Selenite-F enrichment. On the other hand, enrichment was the most helpful method for isolating salmonellae. Studies on cultures from which mixed isolates were obtained indicated that numbers and chance distribution have an effect on the results obtained. The performance of Salmonella-Shigella agar in the isolation of enteric pathogens was inferior, and the effort involved to obtain those isolates was greater than for Hekton enteric and xylose-lysine-desoxycholate agars.

Effective isolation of pathogens for the diagnosis of enteric infection has been a major concern of clinical and public health laboratories for many years. Several media such as bismuth sulfite and brilliant green agars were primarily designed for the isolation of salmonellae. These and other selective media have been too inhibitory for the isolation of shigellae (1, 9), and the nonselective media frequently allow the overgrowth of pathogens by nonpathogens through inhibition or masking of growth. Recent studies indicate that many of the isolates encountered in the clinical laboratory are not isolated from these traditional media (1, 9).

During the past decades, two new media have been developed which seem to have a lower selectivity and a higher rate of recovery than those selective media which were previously available. Xylose-lysine-desoxycholate agar (XLD) was described by Taylor et al. as providing a better rate of isolation of pathogens with fewer false positives needing investigation (6-12). Hekton enteric agar (HE) was developed and was reported to have similar properties which enabled an increased isolation of pathogens with a lower degree of selectivity (3, 4). The studies in which these media were compared with traditional types produced differing results (1, 11); indeed, even divergent results were reported by the same workers at different times (9-12). Conflicting data concerning the performance of Salmonella-Shigella agar (SS) and other enteric plating media, as well as enrichment broths, made it difficult to choose a reasonable combination.

The present study was undertaken to compare the performance of the newer plating media with those in use in the laboratory. The majority of the enteric isolates were Shigella sonnei, and evaluation of their isolation by techniques in use was necessary. The rate of isolation of pathogens, the number of false positive picks, and several variables such as the effects of distribution and numbers on results were observed.

MATERIALS AND METHODS

Specimens. Between 23 May 1972 and 4 June 1973, 455 stool specimens from patients seen at Harborview Hospital were processed for the presence of enteric pathogens. These included 168 specimens from inpatients and the remainder from outpatients. Emulsified stools were plated onto each of several solid media, and equal quantities were inoculated into two enrichment broths.

Media. The plated media used for direct inoculations and subcultures from enrichment broths were MacConkey without crystal violet (BBL) with 2% agar and 0.007% neutral red (MAC), SS, and HE agars. XLD agar was made from XL base (BBL) as recommended by Taylor (6). The MAC and SS were made by the University Hospital media preparation section, and the HE and XLD were made by North-
west Biologicals, Tacoma, Wash. Tetrathionate and Selenite-F broths were provided by Prepared Media Laboratories, Renton, Wash. Examination of tetrathionate enrichment broth was discontinued after the first series of 200 cultures. In the second series, 54 of the 255 cultures were not plated onto XLD due to unavailability of the medium, and SS was discontinued after 118 cultures of this series had been studied. Plated media were evaluated with \textit{Escherichia coli}, \textit{Shigella sonnei}, and \textit{Salmonella enteritidis} belonging to group D. All batches used in this study performed as expected, although the sensitivity of each lot for small numbers was not tested.

\textbf{Culture examination.} After 18 h, several colonies of each type, from all plates, were examined by standard biochemical techniques (2). Plates with a minimum of growth were reincubated for another day. Serogroupings were done by using commercially prepared \textit{Salmonella} and \textit{Shigella} diagnostic antisera (Lederle Laboratories, Pearl River, N.Y.).

\textbf{RESULTS}

The first series of specimens was composed of 200 cultures examined between 23 May 1972 and 28 October 1972, whereas the second consisted of 255 specimens examined from 28 October to 4 June 1973. Tables 1 and 2 show the isolation rates of pathogens as well as the number of false positives which were examined in each series. All 17 strains of \textit{Sh. sonnei} were isolated on MAC in the first series, although one of these did not grow on either XLD or HE on direct plating (Table 1). This occurred even though this strain was isolated from the more inhibitory SS agar from which only 5/17 isolates were obtained. Neither one of the enrichment broths was effective in the isolation of \textit{Sh. sonnei}, regardless of plating or enrichment. No more than 7/17 isolates were obtained from Selenite-F subcultures which were plated on MAC and HE, 5/17 from XLD, and 3/17 from SS. Tetrathionate subcultures revealed no more than 2/17 of these strains. In the second series, no one medium grew all 13 isolates of \textit{Sh. sonnei} (Table 2). One strain was isolated only on HE by direct plating, and another only on XLD.

\begin{table}
\centering
\caption{Yield of pathogens on various media with direct and indirect plating-series 1—200 cultures}
\begin{tabular}{lcccccccc}
\hline
\textbf{Organism} & \textbf{Total} & \multicolumn{4}{c}{\textbf{Direct}} & \multicolumn{4}{c}{\textbf{Tetrathionate}} & \textbf{Selenite-F} \\
 & & \textbf{MAC} & \textbf{SS} & \textbf{HE} & \textbf{XLD} & \textbf{MAC} & \textbf{SS} & \textbf{HE} & \textbf{XLD} & \textbf{MAC} & \textbf{SS} & \textbf{HE} & \textbf{XLD} \\
\hline
\textit{Sh. sonnei} & 17 & 17 & 5 & 16 & 16 & 2 & 0 & 2 & 2 & 7 & 3 & 7 & 5 \\
\textit{Sh. flexneri} & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\
\text{Salmonella B} & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\
\text{Salmonella C} & 4 & 1 & 0 & 1 & 1 & 2 & 1 & 2 & 3 & 3 & 3 & 3 & 4 \\
\hline
\text{False positives} & 35 & 35 & 56 & 43 & 71 & 58 & 79 & 58 & 48 & 40 & 48 & 37 \\
\text{Yield (positive/total picks [%])} & 35 & 15 & 24 & 30 & 7 & 2 & 6 & 8 & 17 & 15 & 20 & 21 \\
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Yield of pathogens on various media with direct and indirect plating-series 2—255 cultures}
\begin{tabular}{lcccccccc}
\hline
\textbf{Organism} & \textbf{Total} & \multicolumn{5}{c}{\textbf{Direct}} & \multicolumn{2}{c}{\textbf{Selenite-F}} \\
 & & \textbf{MAC} & \textbf{HE} & \textbf{XLD} & \textbf{SS} & \textbf{MAC} & \textbf{HE} & \textbf{XLD} & \textbf{SS} \\
\hline
\textit{Sh. sonnei} & 13 & 7 & 12 & 5/7$^c$ & 3/12 & 5 & 7 & 2/8 & 2/12 \\
\textit{Sh. flexneri} & 6 & 0 & 0 & 4 & 6 & NP$^a$ & 0 & 0 & 0 & NP \\
\textit{Salmonella B} & 8 & 1 & 4 & 2/6$^b$ & 0/4 & 8 & 8 & 5/5 & 1/4 \\
\textit{Salmonella C} & 1 & 0 & 0 & 0 & 0 & 1 & 1 & NP & 0 \\
\textit{Salmonella C} & 1 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\
\textit{Salmonella E} & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
\hline
\text{False-positives} & 68 & 66 & 38 & 26 & 58 & 73 & 42 & 23 \\
\text{Yield (positive/total picks [%])} & 11 & 23 & 26 & 10 & 21 & 19 & 16 & 15 \\
\end{tabular}
\end{table}

$^a$ Based on 201 cultures.
$^b$ Based on 118 cultures.
$^c$ Number growing out of number of cultures containing them plated.
$^d$ NP, not plated.
Although only seven cultures from which these pathogens were isolated were plated on XLD, two strains of this series were not isolated on this medium and only 3/12 strains were recovered from SS. Only 7/13 strains were isolated on MAC by direct plating in this series, compared with 17/17 in the previous one. The best results were obtained from direct plating on HE and XLD, from which 12/13 and 5/7 of the isolates were obtained, respectively. All of the isolates were obtained by using just these two media.

The Shigella flexneri isolate in the first series (Table 1) grew on all primary plating media as well as those which were subcultured from Selenite-F. None of the six isolates in the second series were isolated by Selenite-F enrichment. Of this latter group, all six organisms were isolated on XLD by direct plating, 4/6 on HE, and none on MAC. SS agar was not in use at the time these organisms were isolated.

The isolation of all salmonellae, in both series, was enhanced by enrichment with Selenite-F. Without exception, direct plating was not as effective for the recovery of these organisms (Tables 1 and 2). From a total of five strains of Salmonella C₁, only one medium, XLD, demonstrated all of those that were plated (Table 1). The single isolate of the second series was not plated onto XLD. Selenite-F enrichment detected a total of 4/5 on MAC and HE and 3/5 on SS. Tetrathionate was slightly less effective with these salmonellae than was Selenite-F, for one of the isolates present in the same specimen with Sh. sonnei would have been totally missed.

Nine strains of Salmonella B were isolated in the two series. The single isolate in the first series was not found by direct plating, whereas in the second series, direct plating on HE revealed 4/8 strains, 2/6 were isolated on XLD, and none of the four SS-plated were found on that medium (Table 2). One additional strain was found only on MAC on direct plating. The one isolate in the first series was obtained only on MAC and HE after enrichment with Selenite-F, whereas all of the isolates in the second group grew on all of the media to which they were subcultured, except SS from which only 1/4 was isolated (Table 2).

There was considerable variation in the number of false positive colonies picked in the two series. Although SS is a more inhibitory medium than the others, on the whole the number of false positives investigated was not significantly less (Table 1). Proteus accounted for most of the false picks, followed by late lactose fermenting E. coli and Pseudomonas. Tetrathionate broth increased the number of false positives on the plates, whereas Selenite-F did not significantly change the pattern over that obtained by direct plating. If these data are examined from the viewpoint of effort and the resulting yield, with the use of a ratio of the positives obtained to the number of colonies investigated, the results are easier to compare and the usefulness of the medium can be evaluated. In both series it is evident that SS agar ran behind the others in its ability to yield pathogens for the number of suspicious colonies picked. HE and XLD were consistently the most valuable media. Tetrathionate yield rates were very poor, but even with the small number of isolates appropriate for this medium, the yield from subcultures to SS was much lower than for the other plated media (Table 1).

Three mixed infections were uncovered during the course of the study period, and Table 3 shows the distributions of the isolates from each specimen.

A. H. had Sh. sonnei and Salmonella C₁, in his stool. Sh. sonnei was isolated from all four direct plating media and not from any of the Selenite-F subcultures. On the other hand, the

| Identification | Organism                  | Direct         | Selenite     |
|---------------|---------------------------|----------------|--------------|
|               |                           | MAC | HE | XLD | SS | MAC | HE | XLD | SS |
| A. H.         | Sh. sonnei                | 2+  | 1+ | 3+  | 1+ | -   | -  | -   | -  |
|               | Salmonella C₁             |     |    |     |    | 3+  | 3+ | 3+  | -  |
| N. L.         | Salmonella B              | -   | -  | -   | NG*| 3+  | 3+ | 3+  | -  |
|               | Salmonella E              | -   | -  | -   | NG | -   | -  | -   | 2+|
| D. T.         | Salmonella B              | 1+  | NP | -   | NG | 3+  | 3+ | NP  | NG |
|               | Salmonella C₁             | -   | -  | NP  | NG | 3+  | 3+ | NP  | NG |

* Semi-quantitation of growth.
* NG, no growth; NP, not plated.
Salmonella C1 was isolated only from Selenite-F enrichment subcultures and not from the primary plates.

N. L. had two different groups of salmonellae, Salmonella B and E. Salmonella B was isolated from the MAC, XLD, and HE Selenite-F subculture plates, but a prolonged search did not reveal any isolated colonies from SS belonging to this serogroup. The Salmonella E was only on the SS subculture plate, and prolonged searches of the other three media revealed no isolated colonies on that medium which agglutinated in Group B antisera. Confluent growth areas were not searched.

D. T. also had two different serogroups of salmonellae, Salmonella B and C1. The group B organism grew in the first quadrant on the direct MAC plate only, and both the B and C1 grew on MAC and HE from the Selenite-F subculture. This culture was not plated on XLD, and there was no growth at all on SS.

DISCUSSION

From 23 May 1972 to 4 June 1978, 455 stool specimens were examined for enteric pathogens at Harborview Medical Center. Fifty-three pathogens were isolated from these specimens, and 30 of these were Sh. sonnei. This organism accounted for 56% of the pathogens and 81% of the shigellae isolated. These organisms were favored by direct plating where performance of MAC differed greatly in the two series. In the first series all of the isolates were obtained from MAC by direct plating, whereas only 54% of them were isolated on this medium in the second series. The lack of selectivity of MAC makes it possible for lactose fermenters to overgrow or inhibit these organisms. Overall, 80% of the Sh. sonnei which were isolated were revealed on MAC by direct plating, similar to the experiences reported elsewhere (1, 11). HE and XLD performed consistently well with isolation of 90% and 87% of the Sh. sonnei, respectively. All seven of the Sh. flexneri strains were recovered on XLD and 5/7 on HE. The overall performance of XLD in the isolation of shigellae on primary plating was 90%, which is better than that reported by Dunn and Martin (1) and similar to that reported by Taylor and Schelhart (11). HE agar recovered 87% of all the shigellae, and this is also similar to the experience of others (1, 12). All three of these plated media were markedly more effective than SS, from which only 28% of the shigellae tested were isolated. This is a much lower isolation rate than has been reported recently. Dunn and Martin reported that 60% and Taylor and Schelhart indicated that 90% of the shigellae studied were recovered on SS by primary isolation (1, 12). Recent work has shown that R-factor-carrying strains have a low plating efficiency on SS agar (13). It was demonstrated that this property was not directly attributable to the presence of the R-factor for R- segregants also showed this failure. Studies in our laboratories have shown that the plating efficiency of some strains of Sh. sonnei is three logs lower on SS than on XLD and HE (Buhler, unpublished data). Many of the isolates in this study had the same susceptibility patterns and were resistant to more than one antibiotic. They may not only carry R-factors but also be members of the same strains. If it is the property of particular strains to plate poorly on certain media, and that strain predominates in the community, than the problems of isolating organisms from particular media may be accentuated. This may be responsible for the poor showing of SS with Sh. sonnei in these series.

Enrichment for shigellae was less helpful than has been described elsewhere. In this study, only 47% of the isolates were recovered from Selenite-F enrichment, whereas Taylor and Schelhart have reported increasing their yield of shigellae with enrichment (11, 12). Although our experience is more similar to that reported by Dunn and Martin (1), strain differences as well as the other variables described by Taylor and Schelhart (11) may play a role here.

Isolation of salmonellae was greatly enhanced by enrichment with Selenite-F broth and not as well by tetrathionate. The small numbers with which we are dealing here do not give a fair evaluation of tetrathionate. Dunn and Martin have reported increased yields of salmonellae with tetrathionate over Selenite-F and GN broths on all plating media tested (1). This was not the experience of Taylor and Schelhart in whose studies Selenite-F provided the best enrichment for salmonellae (11).

The results obtained are going to be affected by the numbers of organisms present, their chance distribution on the various media, and their inherent ability to grow on a plated medium or multiply in an enrichment broth. Chance distribution was demonstrated in the mixed Salmonella B and E culture. That the Salmonella E grew only on SS in appreciable numbers and the group B organism grew only on the other three media was probably a matter of distribution. Another culture in which Salmonella B was present in small numbers only on a MAC primary plate is probably due to
numbers and distribution. Such randomness of distribution has been described by King and Metzger using replicate plates (4).

We found that the more inhibitory SS medium did not reduce the false positives isolated when compared with the less inhibitory MAC, HE, and XLD. On the basis of yield, SS generated more wasted effort and fewer isolates than did the others. Although there are specific discrepancies between various studies, a common denominator has emerged. All studies, including this work, indicate the superiority of the recently developed HE and XLD media over traditional types. Each of the new media uses a different differential system, and as a result, each can act as a check against the other. The few xylose-fermenting shigellae and _S. enteritidis_ which fail to decarboxylate lysine (2) may be missed on XLD but would be detected on HE. Both media avoid the combination of ingredients which is thought to be responsible for the low plating efficiency of certain strains on SS; i.e., bile salts plus citrate. Ammonium citrates were not tested in this report. For laboratories which must work with a limited number of media, we would concur with Isenberg et al. that one and probably both of these new media should be used for primary screening and subcultures (5). Certainly if a laboratory is using SS as a selective medium, and particularly if the isolation of shigellae is low compared with salmonellae, that medium should be replaced or supplemented by HE or XLD, or both.

In this study, SS agar generated a very low yield of _Sh. sonnei_ isolates as well as other enteric pathogens when compared with HE and XLD. The decreased yield on SS is prominent, and this medium should be considered a poor selective medium compared with the others.

ACKNOWLEDGMENTS
The authors thank the clinical microbiology technologists at Harborview Hospital for their technical assistance and C. George Ray for his helpful suggestions on the manuscript.

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