Enrichment for Plesiomonas shigelloides from Stools

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Bile peptone broth and alkaline peptone water (pH 8.5) were examined as enrichment media for the isolation of Plesiomonas shigelloides from stools, with salmonella-shigella agar as the isolation medium. After 423 parallel examinations in two different experiments, bile peptone broth enrichment for 24 h was observed to be six times more effective (P < 0.01) than direct plating alone on salmonella-shigella agar. Bile peptone broth was found to be twice as effective as alkaline peptone water for the recovery of P. shigelloides from stools.

Plesiomonas shigelloides, a member of the Vibrionaceae, is an aerobic or facultatively anaerobic, gram-negative, nonsporforming rod with lophotrichous flagella. It ferments mannitol with acid but not gas. It is sensitive to O/129 (2,4-diamino-6,7-diisoprolylpteridine) (8) and possesses de-carboxylase and dihydrolase activities. It has been described as an infrequently isolated agent of diarrhea (2) in humans. Taylor and co-workers reported P. shigelloides as one of the agents causing traveler’s diarrhea (6). We are investigating methods for the optimal isolation of P. shigelloides from human and environmental specimens.

A variety of plating media have been used to isolate P. shigelloides. Salmonella-shigella (SS) agar has long been used for the isolation of P. shigelloides (7). However, P. shigelloides isolation on this medium alone has not been totally satisfactory, as SS agar was developed as a selective medium for the isolation of members of the family Enterobacteriaceae. Consequently, several plating media have been described for P. shigelloides isolation, including Plesiomonas differential agar (5), inostitol-brilliant green-bile salts agar (5), and modified SS agar (7).

In addition to direct plating of P. shigelloides, several enrichment media have been proposed. Cooper and Brown (1) examined the utility of gram-negative broth and Rappaport broth as enrichment media for P. shigelloides. They reported that gram-negative broth was a better enrichment medium for this organism than was Rappaport broth (1). Nair and Millership (4) formulated a P. shigelloides enrichment medium called bile salts-brilliant green broth. They compared this enrichment medium with alkaline peptone water (APW) for 219 fecal specimens examined in parallel. They recovered P. shigelloides from only one bile salts-brilliant green broth-enriched specimen. APW failed to recover P. shigelloides from the same specimen. Nair and Millership (4) then artificially seeded normal stools with P. shigelloides and enriched the mixture with bile salts-brilliant green broth containing various concentrations of brilliant green. They reported that bile salts-brilliant green broth containing 0.001 g of brilliant green per liter was superior to APW as an enrichment broth for the isolation of P. shigelloides. Further attempts to define optimal enrichment methods for P. shigelloides were made by Freund et al. (S. M. Freund, J. A. Koburger, W. S. Otwell, and S.-I. Wei, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, P-21, p. 278), who reported that tetrathionate broth without iodine was useful for P. shigelloides enrichment.

We wished to evaluate the use of a single enrichment broth for the isolation of both P. shigelloides and other members of the Vibrionaceae. We selected bile peptone broth (BPB) and APW for comparison, as these media are simple to prepare and widely used.

In the first set of experiments, we used ordinary SS agar as the isolation medium for P. shigelloides, with preplating enrichment in BPB (pH 8.8). BPB enrichment for 6 to 24 h at 37°C has been advocated to improve the isolation of Vibrio cholerae from stool specimens (3). We plated 423 stool specimens on SS agar plates before and after enrichment in BPB for 6 and 24 h. Inoculated plates were aerobically incubated at 37°C. After overnight incubation, typical P. shigelloides colonies (non-lactose fermenting, 1.0 to 2.5 mm in diameter, with a whitish central zone) were tested for the presence of cytochrome oxidase. Oxidase-positive colonies were identified as P. shigelloides by their biochemical properties (8). Using direct plating only, we obtained five P. shigelloides isolates. With a 6-h BPB enrichment, we obtained 9 isolates; with a 24-h BPB enrichment, we obtained 30 P. shigelloides isolates. Isolation rates for P. shigelloides after a 24-h enrichment in BPB were significantly higher than after a 6-h enrichment (P < 0.01) or direct plating (P < 0.01).

In a second set of experiments, we determined the efficacy of APW (pH 8.8) as an enrichment medium for P. shigelloides to be lower than that of BPB. Duplicate rectal swab specimens were collected from 144 patients with diarrhea and were directly inoculated onto SS agar and into BPB and APW at 37°C for 24 h. P. shigelloides was isolated from 2 (1%) directly plated specimens, 6 (4%) APW-enriched specimens, and 12 (8%) BPB-enriched specimens. While the numbers of P. shigelloides isolates were small and insufficient to demonstrate a statistical significance between these media, BPB appeared superior to APW as an enrichment medium for P. shigelloides.

We conclude that the isolation of P. shigelloides from stools on SS agar can be enhanced by the use of 24-h enrichment in BPB. APW, although better than direct plating alone, was less effective than BPB for the recovery of P. shigelloides from stool specimens.

The advantages of BPB include its wide usage, ease of preparation, low cost, and known effectiveness as an enrichment medium for other members of the Vibrionaceae.

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LITERATURE CITED


