Media for the Isolation of Aeromonas hydrophila

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Aeromonas hydrophila has been implicated as an etiologic agent of diarrheal disease (1–3, 8). As more is learned about the ecology and interactions with human illness, more emphasis is being placed on A. hydrophila isolation and identification. The low level of isolations presently reported in most clinical laboratories may not be a true reflection of prevalence or medical significance. This is supported by the fact that isolation rates of Aeromonas sp. frequently increase in laboratories which have taken specific measures to isolate it (4, 7, 10). Perhaps the leading reasons for increased isolation are enrichment coupled with the use of an optimal plating medium for primary isolation (6, 9).

Currently, we are investigating the etiologic agents of diarrheal disease in an ongoing community-based surveillance study in Canto Grande, a low-income suburb of Lima, Peru. We are interested in isolating Aeromonas sp. in both symptomatic and asymptomatic individuals. Our initial isolation methods included the use of the following media: MacConkey agar, salmonella-shigella agar, xylose-lysine-deoxycholate agar, Hektone agar, ceftulodin-irgasan-novobioic agar, thiosulfate-citrate-bile salts-sucrose agar, and Buttler Campylobacter agar (Oxoid, Ltd.). Isolations with these media were coupled with screening of suspicious colonies for their oxidase reaction. Although these media were not specifically designed for the isolation of Aeromonas sp., we encountered a modest number of isolates, i.e., 19 (1.5%) of 1,248 specimens examined during a 6-month period.

We subsequently considered the exclusive use of these media unsatisfactory for primary isolation of Aeromonas sp. for at least three reasons. First, Aeromonas strains are variable in their ability to ferment carbohydrates. Therefore, selecting suspected Aeromonas colonies on the basis of fermentation characteristics can be misleading. Secondly, certain carbohydrates may have an inhibitory effect on the growth of Aeromonas sp. Both xylose and lactose, common in several enteric media, have been reported to be inhibitory for some strains of Aeromonas sp. (6), presumably due to by-products of carbohydrate metabolism. Finally, acid production, a key to clinical differentiation with carbohydrate-containing media, may result in a false-negative oxidase test result. In response to these drawbacks, we sought a reliable screening method for Aeromonas sp. Gracey and co-workers recommended the use of sheep blood agar with 10 μg of ampicillin per ml (ASBA) (4). We began using this medium, preceded by enrichment with alkaline peptone water (APW) (pH 8.4) (6, 9) and obtained a markedly increased isolation rate.

Stool specimens were transported to the laboratory within 2 h of collection on swabs in modified Amies (clear) transport medium (Precision Scientific, Inc.). The plating media previously described were inoculated directly. In addition, APW was inoculated and incubated for 16 to 18 h at 35°C, after which a large (3-mm) loopful from the upper one-third of the tube was streaked on ASBA. Plates were incubated at 35°C for 18 to 24 h and were examined for low, gray, moist-appearing colonies with or without hemolysis. Individual colonies were tested for oxidase activity. Oxidase-positive colonies were identified as A. hydrophila by the API 20E (Analytab Products) system, sensitivity to 0/129 vibriostatic agent (2,4-diamino-6,7-diisopropylpteridine), and their ability to grow in various concentrations (0, 3, 7, or 10%) of sodium chloride.

After the introduction of APW enrichment and ASBA agar, our isolation rates increased dramatically (Table 1). After nearly 1 year of use, during which we examined 2,386 stool specimens, our overall isolation rate increased to 15.3% (366 Aeromonas isolates). The majority of isolations (67.5%) were made exclusively from APW-ASBA media, while the remaining isolations were made from other media alone (14.2%) or isolated on both the APW-ASBA plate and other media (18.3%). Of the 366 stools yielding Aeromonas isolates, 265 (72.4%) yielded only A. hydrophila and none of the following etiologic agents: Salmonella spp., Shigella spp., Yersinia spp., Plesiomonas spp., Campylobacter jejuni, Vibrio spp., enteropathogenic and enterotoxigenic Escherichia coli, or rotavirus. Of these, 191 (72%) were from diarrheal stools, while 74 (28%) were from normal stools.

Before we began using APW-ASBA, we isolated Aeromonas sp. from only 1.5% of specimens. After the introduction of APW-ASBA, we isolated Aeromonas sp. from 5.0% of specimens (119 of 2,386 cultures) with the same media previously used, exclusive of APW-ASBA. In other words, we experienced an increased isolation rate not related to the use of APW-ASBA. This increase in Aeromonas isolations was probably due to our increased proficiency at identifying Aeromonas colonies from conventional media. We found that the liberal use of the oxidase test (with confirmation of preliminary results) and careful attention to colony morphology led to increased rates of Aeromonas isolations with conventional enteric media. However, increased proficiency alone is insufficient to explain the marked improvement with APW-ASBA. Our Aeromonas isolation rate with APW-ASBA (314 of 2,386 [13.2%]) was 2.6 times that with...
The marked difference in the number of Aeromonas isolations from various media (Table 2) caused us to examine the growth and colony characteristics of this organism on each medium we used. Therefore, we randomly selected 112 of our strains, irrespective of the medium of isolation, and grew them overnight in Mueller-Hinton broth. We then diluted the broth cultures with phosphate-buffered saline to equal a 0.5 McFarland turbidity standard (approximately $10^8$ to $10^9$ cells per ml). One drop of diluted culture was spread onto each of the media to be tested. Plates were incubated at 35 ± 1°C for 18 to 24 h and examined for growth characteristics and colony appearance. Aeromonas colonies resembling those of organisms for which the medium was designed to differentiate or select were designated suspicious, i.e., colonies which would likely be selected for further identification as, for example, lactose-nonfermenting colonies on MacConkey agar.

Nearly all strains grew on the media tested with the exception of thiosulfate-citrate-bile salts-sucrose and cefsulodin-irgasan-novobiocin (Table 3). However, Aeromonas colonies did not uniformly appear as suspicious on the respective media and would probably have been missed in an actual screening procedure. In contrast, Aeromonas colonies on ASBA were low, gray, moist, and consistently oxidase positive.

Hemolytic activity, as previously reported (2,5), was observed with only approximately 20% of isolates. Of 366 Aeromonas isolates grown on ASBA medium, 53 (14%) were beta-hemolytic, while 22 (5.8%) were alpha-hemolytic.

Enrichment with APW for various times and incubation temperatures has long been advocated for members of the family Vibrionaceae. Accordingly, APW has been found to be a useful enrichment medium for Aeromonas sp. (6, 9). However, extending the incubation time beyond 18 h has been associated with a marked reduction in isolation rates with this medium (6). It should be noted that, in this study, APW enrichment was used only with ASBA and TCBS plates; therefore, it was not possible for us to evaluate its effect on Aeromonas isolation rates from the other media we used.

In summary, our experience with several commonly used enteric media gives further evidence of their inadequacy for the recovery of A. hydrophila from stool specimens. We found that the combination of APW enrichment with primary plating on ASBA significantly increased our isolation of Aeromonas sp. from stool samples of both symptomatic and asymptomatic individuals. We felt this increase was due to the enriching action of APW and the unique ability of ASBA to support the growth of Aeromonas sp., yield an accurate oxidase test result, present characteristic colonies, and not rely on fermentable carbohydrates for colonial differentiation.

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**LITERATURE CITED**