Growth of Aeromonas Species on Enteric Agars

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The efficacy of eight routine enteric agars for supporting the growth of 32 strains of Aeromonas spp. (17 A. hydrophila strains, 8 A. sobria strains, and 7 A. caviae strains) was investigated. The plating efficiency of Aeromonas spp. on these media varied greatly (range, 0 to 100%), as did their colony size when compared with that on noninhibitory medium (5% sheep blood agar). Plating efficiency on seven of these eight media appeared to be strain- and not species dependent. Overall, eosin-methylene blue and Hektoen enteric agars showed low plating efficiencies for A. hydrophila, whereas both A. sobria and A. caviae were severely inhibited on brilliant green agar. When all these species are considered collectively, deoxycholate, MacConkey, and xylose lysine deoxycholate appeared to be the most satisfactory routine agars for Aeromonas spp. recovery when used in conjunction with blood agar.

Aeromonas species are increasingly being recognized as an important cause of gastroenteritis worldwide, and it has recently been suggested that they are also a cause of traveler’s diarrhea (5, 6, 9). Evidence supporting these aquatic bacteria as important gastrointestinal enteric pathogens includes well-described case studies (1, 3, 4, 13, 14), a large case-controlled survey (5), the demonstration of enterotoxin-producing strains (2), serologic responses subsequent to gastrointestinal infection (3, 13), and resolution of symptomatology subsequent to antimicrobial therapy directed against Aeromonas spp. (1, 4, 13).

Although Aeromonas species are generally accepted as a cause of bacterial gastroenteritis, the actual incidence of this disease as well as colonization rates in humans may vary widely with geographic locale. Although a number of selective agars or enrichment broths have been devised for the recovery of these organisms (17), the clinical significance of the recovery of Aeromonas spp. from such agars is unknown because growth obtained on such media may reflect only transient colonization, particularly during the warmer months. In addition, the use of a selective agar for the recovery of Aeromonas spp. as part of a routine gastrointestinal workup may be costly and time-consuming.

Recently, Holmberg and Farmer suggested that the isolation of Aeromonas spp. from stools be reported only when moderate-to-heavy growth occurs on primary plating (7). However, moderate-to-heavy growth of Aeromonas spp. from stools can be expected only if the media used are not inhibitory to Aeromonas spp. growth. For this reason, we decided to evaluate the growth of Aeromonas spp. on enteric media widely used for the isolation of Salmonella, Shigella, and other enteric pathogens.

MATERIALS AND METHODS

Bacterial strains and culture media. The 17 strains of Aeromonas hydrophila, 7 strains of Aeromonas caviae, and 8 strains of Aeromonas sobria used in this study were isolated from clinical specimens submitted to the Mount Sinai Hospital laboratories. Isolates were initially identified to the genus level by established criteria (16) and then identified to the species level as previously described (8, 11).

Brilliant green (BG), bismuth sulfite (BS), deoxycholate (DC), Hektoen enteric (HE), salmonella-shigella (SS), and blood agar (BA) base (heart infusion agar) agars were obtained from Difco Laboratories, Detroit, Mich. MacConkey (MAC) agar was obtained from GIBCO Diagnostics, Madison, Wis., and xylose lysine DC (XLD) and Levine's eosin-methylene blue (EMB) agars were obtained from BBL Microbiology Systems, Cockeysville, Md. BA plates contained 5% sheep blood. All of the enteric agars and the BA plates were prepared in the microbiology laboratory, Bronx Veterans Administration Medical Center.

Measuring plating efficiency. A method similar to that described by Marold et al. (12) was used to measure plating efficiency. Organisms were grown overnight at 37°C in Trypticase soy broth (BBL). Dilutions of each organism were made in Trypticase soy broth to 10⁻³, 10⁻⁶, and 10⁻⁷. A 0.1-ml sample of each dilution was plated on the eight enteric agars and BA. After 48 h of incubation at 37°C, colonies were counted on BA plates. Colony counts were made and colony sizes were measured on the set of plates which yielded a colony count on BA of 30 to 300. Plating efficiency was expressed by dividing the number of colonies on the enteric agar by the number of colonies on the BA plate and multiplying by 100%. Mean plating efficiency for a medium was obtained by adding the number of colonies of all the strains on the enteric agar, dividing by the total number of colonies on the corresponding BA plates, and multiplying by 100%.

RESULTS

There was a wide variation in plating efficiency among strains of the same Aeromonas sp. on the various media (Fig. 1). On all media tested, some Aeromonas isolates were significantly inhibited (1-log reduction, 90% decrease in CFU) compared with counts obtained on BA. This inhibition was random in nature, that is, one strain did not usually
show significant reduction in CFU on more than two enteric agars.

The relative inhibition of the 32 Aeromonas isolates on the eight media tested is shown in Table 1. DC agar was the least inhibitory medium tested, with only 9.4% (3/32) of the Aeromonas strains inhibited more than 90% in comparison with BA. In fact, of all the media tested, only DC and XLD permitted the growth of all 32 strains tested to some extent. Conversely, BG agar was most inhibitory to Aeromonas spp., with 59% (19/32) of the strains tested showing a 2-log reduction (99%) in CFU on this medium. BG agar failed to support the growth of any A. caviae or A. sobria strains (Table 1).

Individual plating efficiencies and mean colony sizes for each of the three Aeromonas spp. are listed in Table 1. Three media (BG, BS, and EMB) yielded small mean colony sizes (1.0 to 2.0 mm) for all three Aeromonas spp., while MAC, DC, and XLD exhibited the largest Aeromonas colony sizes after 48 h of incubation. Many Aeromonas strains produced colonies on media regarded as highly selective for Salmonella and Shigella spp. (BS and SS). Of 32 strains tested, 22 (69%) showed less than a 1-log reduction in CFU on BS, while 21 (66%) showed a similar phenomenon on SS.

**DISCUSSION**

The number of proven bacterial gastroenteritis agents has increased over the past several years and now includes such diverse groups as certain serotypes of Yersinia enterocolitica, Campylobacter spp., invasive Escherichia coli (O:157, H:7), Plesiomonas shigelloides, new halophilic Vibrio spp., and Aeromonas spp. Because the development of enteric agars, essentially designed for the primary isolation of Salmonella and Shigella spp., predated the discovery of these bacteria, either new selective agars are required to isolate these microorganisms (as in the case of Campylobacter spp.) or laboratory workers must make use of existing selective and differential agars. From this study, it is apparent that one factor potentially influencing the incidence of Aeromonas gastroenteritis seen in different geographic locales is which enteric media is used in each laboratory. Media such as EMB, BG, and BS are generally unsatisfactory for the recovery of Aeromonas spp., owing to the poor plating efficiency and small colony size of Aeromonas spp. on these agars. Other common enteric media, such as HE and XLD, are unsuitable because they contain sucrose; Aeromonas spp., generally sucrose positive (85%), are overlooked on these media. In fact, in our experience, lactose-positive Aeromonas isolates are not uncommon and thus would be similarly missed on MAC agar (8, 11). Because individual strains show large variations in relative inhibition on different enteric media, it seems preferable that at least two different agars be used in the attempted isolation of Aeromonas spp.

Holmberg and Farmer recommended that the presence of Aeromonas and Plesiomonas organisms in stool specimens be reported when they predominate or when their growth is heavy on nonselective media (7). The data reported here suggest that Aeromonas spp. sometimes do not predominate on enteric media because of growth inhibition of some strains on these media. To evaluate whether Aeromonas spp. growth from a stool specimen is heavy or predominant appears to require a BA plate or other nonselective, nonenteric media. All of the enteric media used in this study, including those regarded as nonselective, inhibited the growth of some Aeromonas strains. Other researchers previously recommended the routine use of BA in the detection of Aeromonas spp. in feces (6, 9, 10, 15). Our data support this recommendation. However, because swarming Proteus spp. on BA can prevent the isolation of Aeromonas spp., the enteric agars are an essential adjunct to the use of BA.

Because cost limits the variety of media that can be used for the workup of fecal specimens, many laboratories will continue to rely on BA and enteric media for the isolation of Aeromonas spp. Among the enteric media used for this purpose, DC appears to be least inhibitory for Aeromonas spp. When selecting media to be used for the routine workup of feces, it is well to bear in mind that 11 of 32 Aeromonas strains (34%; Table 1) were inhibited more than 90% on the widely used HE and EMB media. It is therefore appropriate that enteric agars selected for routine use in the workup of gastrointestinal specimens not only be able to isolate Salmonella and Shigella spp. satisfactorily but be flexible enough to recover other potential enteric pathogens of medical importance. If the frequency of Aeromonas diarrhea in the patient population of a given institution is high, the routine use of a selective agar specifically designed for Aeromonas spp. with a relatively high plating efficiency for most strains would seem to be advantageous. This study reports the plating efficiency of Aeromonas clinical isolates on commonly used enteric agars. The plating efficiency of these

**TABLE 1. Plating efficiency and colony size of Aeromonas species on enteric agars**

<table>
<thead>
<tr>
<th>Medium</th>
<th>A. hydrophila</th>
<th>A. caviae</th>
<th>A. sobria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPE (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MPE (%)</td>
<td>MCS (mm)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BG</td>
<td>0–100</td>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>BS</td>
<td>0–100</td>
<td>41</td>
<td>2.0</td>
</tr>
<tr>
<td>DC</td>
<td>3–100</td>
<td>51</td>
<td>3.5</td>
</tr>
<tr>
<td>EMB</td>
<td>0–77</td>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>HE</td>
<td>9–88</td>
<td>28</td>
<td>3.5</td>
</tr>
<tr>
<td>MAC</td>
<td>0–100</td>
<td>31</td>
<td>3.5</td>
</tr>
<tr>
<td>SS</td>
<td>0–77</td>
<td>25</td>
<td>2.5</td>
</tr>
<tr>
<td>XLD</td>
<td>1–100</td>
<td>42</td>
<td>3.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> BG, Brilliant green; BS, bismuth sulfite; DC, deoxycholate; EM, Levine’s eosin-methylene blue; HE, Hektoen enteric; MAC, MacConkey; SS, salmonella-shigella; XLD, xylose lysine deoxycholate.

<sup>b</sup> RPE, Range of plating efficiency.

<sup>c</sup> MPE, Mean plating efficiency.

<sup>d</sup> MCS, Mean colony size.
agars with *Aeromonas* spp. directly from stool specimens remains to be determined.

LITERATURE CITED