Enhanced Recovery of *Plesiomonas shigelloides* following an Enrichment Technique

SUSAN M. FREUND, JOHN A. KOBURGER and CHENG-I WEI*

Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611

(Received for publication July 6, 1987)

**ABSTRACT**

Enrichment techniques using five broths (gram-negative broth, alkaline peptone water, tetrathionate broth without iodine and two *Plesiomonas* broths) were compared to direct plating methods using freshwater samples to determine their ability to increase the isolation rate of *Plesiomonas shigelloides*, a suspected food and waterborne pathogen. Tetrathionate broth consistently gave significantly (*p*<0.05) greater recovery of *P. shigelloides* than the other four broths tested as well as by direct plating. Incubation of the enrichment broths at 40°C also resulted in significantly higher recovery of *Plesiomonas* than at 35°C. It is therefore suggested that for routine monitoring of *shigelloides*, tetrathionate broth incubated at 40°C be used for enrichment before plating.

*Plesiomonas shigelloides* is a gram-negative, non-sporforming bacillus belonging in the family *Vibrionaceae* (21). It has been isolated from a variety of sources, including soil, water, food, wild and domestic animals as well as diarhheaic and asymptomatic humans (1,2,4,24). This organism is generally considered to be a waterborne agent, found most commonly in fresh, surface waters (1,9,21).

*P. shigelloides* has been linked to a number of cases of gastroenteritis that involved consumption of contaminated water or food such as raw fish or oysters (3,5,12,19). One such outbreak occurred in Florida in 1983. In a 6-d period, 29 people who had eaten raw oysters were affected with gastroenteritis (10).

*Plesiomonas* is thought to be an opportunistic pathogen. A person’s age and general health at the time of exposure can determine the occurrence as well as the severity of the disease. The very young, old and infirm seem to be those segments of the population at greatest risk (1,4,13,16,18). To date, though, there has been varied success in demonstrating the pathogenicity of *P. shigelloides* under laboratory conditions (8,11); most of the evidence linking it to disease is epidemiological (7,9,17).

The isolation techniques available at the present time are mainly limited to direct plating methods. Direct plating often does not detect low numbers of this organism. Many media used for isolation of *Salmonella* and *Shigella* are also used for *Plesiomonas* isolation. However, these media are often unsuitable because they contain agents toxic to *Plesiomonas* (2,4,13,16,17,21).

Miller and Koburger in 1986 (14) compared two solid plating media, *plesiomonas* agar (PL; 14) and inositol brilliant green bile salts agar (IBB; 21), for recovery of *Plesiomonas*. These two media are formulated specifically for *Plesiomonas* and they are recommended for isolating this organism from environmental samples. Both media are suggested because the recovery of this organism from the samples was highest using IBB; but in laboratory controlled studies, the PL agar was less inhibitory for injured organisms (14).

Incorporation of an enrichment procedure may increase the isolation of *shigelloides* from various samples. Thus in this study, five enrichment media were compared to the direct plating method described by Miller and Koburger (14). The five enrichment broths were: alkaline peptone water (APW), gram-negative broth (GN), tetrathionate broth (TT), *plesiomonas* enrichment broth (PLE) and a modified *plesiomonas* enrichment broth (mod PLE). Development of a more sensitive isolation procedure could enhance detection of *Plesiomonas* in samples where it may have previously been unnoticed. This will lead to a better understanding of this organism’s ecology as well as survivability and resuscitation in both environmental and clinical situations.

**MATERIALS AND METHODS**

Water samples

Water samples were collected from five freshwater sites in Gainesville, FL. Samples 1, 3 and 4 were collected from eutrophic recreational lakes. Sample 3 was obtained from a creek receiving urban runoff and Sample 5 was from a less eutrophic, shallow pond. These samples were collected in sterile Whirl-Pak bags (Fisher Scientific, Orlando, FL) and transported immediately to the labo-
TABLE 1. Evaluation of enrichment broth, agar plating media and incubation temperature for recovery of Plesiomonas shigelloides (% of total colonies).

<table>
<thead>
<tr>
<th>Broth</th>
<th>PL 35°C</th>
<th>PL 40°C</th>
<th>IBB 35°C</th>
<th>IBB 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>GN</td>
<td>1.20</td>
<td>1.60</td>
<td>0.03</td>
<td>0.20</td>
</tr>
<tr>
<td>APW</td>
<td>0.81</td>
<td>2.04</td>
<td>14.52*</td>
<td>6.88</td>
</tr>
<tr>
<td>PLE</td>
<td>0.04</td>
<td>2.32</td>
<td>1.40</td>
<td>2.64</td>
</tr>
<tr>
<td>PLE mod</td>
<td>0.20</td>
<td>0.00</td>
<td>0.17</td>
<td>1.76</td>
</tr>
<tr>
<td>TT</td>
<td>21.12*</td>
<td>15.68*</td>
<td>68.92*</td>
<td>53.96*</td>
</tr>
</tbody>
</table>

*Difference significant between broths at 0.05 level as determined by the LSD t-test.

**Difference significantly higher than GN, PLE and mod PLE but significantly lower than TT at 0.05 level.

N=25 environmental water samples.

TABLE 2. Comparison of direct plating and tetrathionate enrichment for recovery of Plesiomonas shigelloides.

<table>
<thead>
<tr>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct plating</td>
<td>36.0</td>
<td>3.6</td>
<td>26.2</td>
<td>25.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Enrichment*</td>
<td>76.6</td>
<td>64.8</td>
<td>81.8</td>
<td>68.0</td>
<td>86.6</td>
</tr>
</tbody>
</table>

*The values of the enrichment group are significantly higher (p<0.05) than the direct plating group as determined by the LSD t-test.

**The water samples were serially diluted using Butterfield's phosphate buffer (22) before being surface inoculated onto IBB and PL agars. The plates were incubated at 35°C for 24-48 h and counts were made of typical colonies presumed to be Plesiomonas.

Enrichment procedure

Ten ml of each water sample was inoculated in duplicate into 90-ml portions of each of the five test broths. After mixing, one-half of the duplicate broth samples were incubated at 35°C and the other half at 40°C for 18 h. The enriched samples were then streaked onto both PL agar and IBB agar plates and incubated at 35°C for 24-48 h. Presumptive counts of typical Plesiomonas colonies were done and the total number of colonies was counted. The relative rate of recovery of Plesiomonas was expressed as the ratio of total Plesiomonas colony counts to the total bacterial colony numbers on the plates.

Direct plating procedure

The five enrichment broths included: gram-negative broth (GN, Difco), alkaline peptone water (APW, 22), tetrathionate broth without addition of iodine (TT, Difco), a plesiomonas enrichment broth (PLE) and a modified plesiomonas enrichment broth (mod PLE). Both PLE and modified PLE were developed in our laboratory. The PLE is comprised of ammonium nitrate 0.1%, inositol 0.2%, yeast extract 0.05%, potassium phosphate 0.5%, magnesium sulfate 0.02% and bile salts 0.1%. The modified PLE is the same as PLE except that the amount of inositol is reduced to 0.1%. The pH is adjusted to 7.6 with 0.1 M NaOH for both PLE and mod PLE.

Confirmation

Presumptive Plesiomonas isolates obtained from broth enrichment and direct plating were confirmed by inoculating the isolates on Triple Sugar Iron agar slants (TSI, Difco) and Inositol Gelatin Deeps (14). An oxidase test was also done. Plesiomonas is alkaline over acid on TSI and does not produce gas. It is able to ferment inositol, but cannot hydrolyze the gelatin in the Inositol Gelatin Deeps. It is oxidase-positive (14,21).

Statistical analysis

A 5 x 3 factorial design analysis of variance using the General Linear Models Procedure in the Statistical Analysis System (19) was applied to compare the relative effectiveness of the broths in enhancing recovery of P. shigelloides. Significant differences between broths were determined by the Least Significant Difference (LSD) t-test.

RESULTS AND DISCUSSION

Recoveries of Plesiomonas on IBB and PL agars for each of the five test broths are shown in Table 1. The values range from 0.04 to 69% of the total colonies being Plesiomonas. Comparison of the five enrichment broths revealed tetrathionate to be significantly (p<0.05) superior to all others tested, either at the two different incubation temperatures or on the two isolation media. The only other broth that gave a significantly higher recovery rate was the alkaline peptone water (APW) at 40°C on plesiomonas agar, but this value was still significantly (p<0.05) lower than that of tetrathionate under the same conditions. Other researchers have noted some success using tetrathionate enrichment. VanDamme and Vandepitte (23) reported that tetrathionate broth without iodine was superior to tetrathionate with iodine.
as an enrichment step before plating on salmonella-shigella agar, although they indicated that direct plating on MacConkey's agar achieved a higher isolation rate. Alkaline peptone water is also cited in a number of references for Plesiomonas enrichment (4, 16), but again these workers recommended direct plating over enrichment.

It is noteworthy that generally IBB appeared to be a better medium for recovery of Plesiomonas when the numbers of Plesiomonas in the samples were low. This was true with GN, APW, PLE and mod PLE. When P. shigelloides was present in greater numbers as was true following tetrathionate enrichment, plesiomonas agar proved more effective at isolating this organism at both 35°C and 40°C. This may be attributed to the greater amount of bile salts in the IBB agar as well as the ability to recognize Plesiomonas colonies on PL agar. A higher concentration of bile salts helps to reduce the level of some of the competing bacteria and this may allow a greater chance of recovering the Plesiomonas colonies that may be present (16).

The elevated incubation temperature of 40°C was superior overall in enhancing recovery of Plesiomonas. The inhibition of some of the competing microorganisms such as Pseudomonas and some of the Enterobacteriaceae which are often found in food and water may have contributed to this higher recovery rate (6, 9, 14, 15). Miller and Koburger (15) reported that of 40 Plesiomonas isolates tested, all were able to grow at 40°C, but that only 25% grew at 45°C. For environmental samples, therefore, it is suggested to incubate enrichment cultures no higher than 40°C to avoid the risk of inhibiting some Plesiomonas.

The advantage of using the tetrathionate enrichment technique to enhance the detection of Plesiomonas is well demonstrated by data in Table 2. The value of enrichment is not so great for a highly eutrophic sample such as Sample 1 because by using direct plating only, about 35% of the colonies are easily detected as Plesiomonas. However, in a less eutrophic sample such as Sample 5, where only 3% of the colonies are Plesiomonas as shown by the direct plating method, the benefits of this enrichment step become obvious; the recovery rate for Plesiomonas was increased to 86% post-enrichment. The chances of finding a Plesiomonas colony when only 3% of the total colonies are Plesiomonas is low, but it can hardly be missed when it is present as 86% of the colonies. It should also be noted that in 12% of the samples tested, no Plesiomonas colonies could be detected by direct plating and yet it was easily isolated following enrichment. It is under such conditions, when the organism is present in low numbers, that the enrichment technique can be a significant aid in the study of the ecology of this microorganism. Enhancement of Plesiomonas detectability in food and environmental samples will help to better understand this organism and its ecological significance.

REFERENCES