Rapid Isolation of *Yersinia* spp. from Feces

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Direct plating or cold enrichment or both have been used to isolate *Yersinia* spp. from feces. Freeze-shock double enrichment and KOH treatment have been recommended for recovery of *Yersinia enterocolitica* from surface waters and food, respectively. These techniques were evaluated as alternative methods for rapid recovery of *Yersinia* spp. from feces. Stool samples were homogenized in buffered saline and autoclaved. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were each added to the suspension at a final concentration of $1.5 \times 10^6$ colony-forming units per ml. *Yersinia* cells were then added to a final concentration of $1.5 \times 10^5$, $1.5 \times 10^4$, $1.5 \times 10^3$, or $1.5 \times 10^2$ colony-forming units per ml. A total of 21 strains of *Y. enterocolitica*, 2 of *Yersinia kristensenii*, and 1 each of *Yersinia intermedia* and *Yersinia fredriksenii* were tested. For freeze-shock double enrichment, seeded stool samples were frozen overnight ($-70^\circ$C), transferred successively to *m*-tetrazionate broth (6 h, $37^\circ$C) and selenite broth (2 h, $37^\circ$C) and plated on MacConkey, salmonella-shigella, and cellobiose-arginine-lysine agars for quantitation. For KOH treatment, seeded stool samples were mixed with 0.5% KOH at a ratio of 1:2 for 2 min and plated as described above. *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were virtually eliminated after either method was used. All *Yersinia* strains were recovered after KOH treatment even at the lowest initial concentration ($1.5 \times 10^3$ colony-forming units per ml). However, after freeze-shock double enrichment, not all strains were retrievable, and those isolates which were recovered were grown only from samples containing the highest number of *Yersinia* strains ($1.5 \times 10^6$ colony-forming units per ml). KOH treatment of stool samples seems to be a viable substitute for more protracted methods of recovering *Yersinia* spp.

Although certain *Yersinia* serotypes or biotypes or both can be recovered by direct plating of feces on appropriate media (8, 12), others (which may be present in smaller numbers) are not generally isolated unless cold enrichment techniques are employed (5, 6, 12, 14). Cold enrichment is of limited clinical value, however, as the organism may not be recovered for up to 3 weeks after inoculation of the specimen. Recently, several other, more rapid techniques have been used to isolate *Yersinia enterocolitica* from the environment and foodstuffs. One of these techniques, freeze-shock double enrichment (G. A. Burton and R. M. Cody, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C170, p. 305), is recommended for the recovery of *Y. enterocolitica* from surface waters; another, alkali treatment, is recommended for rapid recovery of *Y. enterocolitica* from food (1). This report describes our experiences in trying to adapt these two procedures for rapid recovery of *Yersinia* spp. from stool samples.

MATERIALS AND METHODS

*Yersinia* strains. A total of 25 strains of various *Yersinia* species were used in this study. Of these, 2 strains of *Yersinia kristensenii*, 1 strain each of *Yersinia intermedia* and *Yersinia fredriksenii*, and 12 strains of *Yersinia enterocolitica*, including 1 O:1,2a,3 biotype 3, 1 O:2a,2b,3 biotype 2, 1 O:3 biotype 4, 1 O:4,32 biotype 1, 1 O:5 biotype 1, 1 O:5,27 biotype 1, 1 O:6,32 biotype 1, 1 O:7,8 biotype 1, 1 O:8 biotype 2, 1 O:8,19 biotype 1, 1 O:9 biotype 2, and 1 O:10 biotype 1, were obtained from D. J. Brenner, Centers for Disease Control, Atlanta, Ga. The remaining nine *Y. enterocolitica* strains, including one O:5 biotype 1, two O:5a biotype 1, three nontypable biotype 1, one O:7,8 biotype 1, one O:6 biotype 1, and one O:6,30 biotype 1, were isolated at the Jewish Hospital of St. Louis from adult patients with gastrointestinal illnesses. Serotyping of the clinical strains was performed by S. Toma, Laboratory Services Branch, Ministry of Health, Toronto, Ontario. Biotyping was performed at The Jewish Hospital of St. Louis with the well-known schema of G. Wauters (G. Wauters, Ph.D. thesis, Catholic University, Louvain, Belgium, 1970) (Table 1).

Media. MacConkey (Mac), salmonella-shigella (SS),...
TABLE 1. Biotyping of Yersinia strains

<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithinase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>+   a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose (acid)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose (acid)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trehalose (acid)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ornithine dehydrogenase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a This reaction was delayed.
b Test performed at 25°C.

and cellobiose-arginine-lysine (CAL[4]) agars and selenite and m-tetrathionate broths were purchased from Remel Regional Laboratories, Lenexa, Kans.

Weak alkali solution. Potassium hydroxide (KOH) pellets (Fisher Scientific Co., Fair Lawn, N.J.) were weighed and dissolved in 0.85% NaCl to give a stock solution of 40% KOH; this solution was filter sterilized and stored at 4°C. A 1:80 dilution of the stock solution was made in 0.85% NaCl to give a working solution of 0.5% KOH.

Experimental protocol. Feces were homogenized in phosphate-buffered saline and autoclaved. Separate suspensions (3 x 10^7 colony-forming units [CFU/ml]) of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa were prepared in 0.85% NaCl. Portions (20 μl) of each suspension were then added to 1 ml of the autoclaved fecal homogenate to give a final concentration of 1.5 x 10^6 CFU/ml; the fecal homogenate was then blended vigorously in a Vortex mixer to distribute the organisms. Yersinia cells were then added to a final concentration of 1.5 x 10^3, 1.5 x 10^4, 1.5 x 10^5, or 1.5 x 10^6 CFU/ml. Portions of seeded stool samples were plated on Mac, SS, and CAL agars for quantitation. This served as a control to evaluate the actual numbers of the various organisms in the seeded specimens.

For freeze-shock double enrichment, portions of the seeded stool samples were frozen overnight at -70°C, transferred successively to m-tetrathionate broth (37°C for 6 h) and selenite broth (37°C for 2 h), and quantitatively plated on Mac, SS, and CAL agars.

For the alkali treatment method, seeded stool samples were mixed with 0.5% KOH at a ratio of 1:2, blended vigorously in a Vortex mixer for 2 min, and plated as described above.

All isolation plates were incubated at 37°C for 24 h and then left at room temperature for an additional 24 h. Pinpoint colonies of Yersinia spp. were visible after 24 h at 37°C and developed typical colonial morphology by 48 h, when the incubation temperature was changed to 25°C. Presumptive oxidase-negative colonies of Yersinia spp. were identified with the API 20E system (Analytab Products, Plainview, N.Y.).

RESULTS

The results of freeze-shock double enrichment of seeded stool samples are shown in Table 2. Recovery of Yersinia spp. was best accomplished on Mac agar; growth of strains on CAL agar closely paralleled that on Mac agar, but growth on SS agar was extremely variable. Only 20 strains (80% of those tested) were recovered (on Mac agar) by freeze-shock double enrichment when the bacteria were present at the highest initial concentration (1.5 x 10^6 CFU/ml). Moreover, the lower the initial concentration of the bacteria, the fewer the strains grown; only four strains were isolated (on CAL agar) when the bacteria were present at an initial concentration of 1.5 x 10^5 CFU/ml. The growth of E. coli, K. pneumoniae, and P. aeruginosa was greatly inhibited by freeze-shock double enrichment, although occasional breakthrough flora appeared on the agar plates.

The results obtained after KOH treatment of seeded stool samples are shown in Table 3. Again, the greatest number of strains was recovered on Mac agar; CAL agar was almost as effective, but SS agar was definitely inhibitory to a large number of Yersinia strains. All Yersinia isolates were recovered (on Mac agar) after KOH treatment even at the lowest initial concentration of bacteria (1.5 x 10^3 CFU/ml). Furthermore, E. coli, K. pneumoniae, and P. aeruginosa were completely eliminated.

DISCUSSION

Cold enrichment has been the method of choice for recovery of Yersinia spp. from stool samples. This technique is of particular value in recovering Y. enterocolitica from asymptomatic carriers and convalescent patients (8). Recently, a report by Van Noyen et al. (13) suggested that strains which are recovered only by cold enrichment (especially biotype 1) may not cause diarrheal disease, and that, therefore, there is no need to use cold enrichment methods for isolation of pathogenic Yersinia spp. However, biotype 1 strains have been implicated as etiological agents of gastrointestinal illness in Canada (7), South Africa (9), the United States (2, 14), and Belgium (12). Furthermore, all of the isolates that we have recovered at The...
of St. Louis have been biotype 1 strains, and all seemed to be involved in the disease process. None of these isolates was recovered by direct plating methods which, like cold enrichment, included setting up duplicate room temperature and 35°C plates. We therefore suggest that until more data on the pathogenicity of biotype 1 strains are accumulated, cold enrichment continue to be used to recover Yersinia spp. (15).

Unfortunately, cold enrichment is of limited clinical value because of the extended time involved between receipt of the specimen and isolation of the organism. Therefore, this study was undertaken to assess the feasibility of using two more rapid techniques for recovery of Yersinia spp. from feces. While freeze-shock double enrichment did not prove to be a valuable method for recovery of Yersinia spp. from stool samples, KOH treatment of feces seems to be a viable alternative to cold enrichment. Clinical studies are now under way in a number of laboratories, including our own, to compare KOH treatment of stool samples with cold enrichment; recently, workers in Montreal have successfully recovered Y. enterocolitica from the feces of ill and convalescent children using this technique (C. Pai, personal communication).

KOH treatment has several advantages over cold enrichment. The technique allows the isolation of small numbers of Yersinia spp. in pure culture after only 48 h, as opposed to up to 3 weeks for cold enrichment, and weekly subcultures are not needed. Thus, a microbiologist would be able to report the isolation of Yersinia spp. within the same time frame currently needed for notifying a physician of infection due to Salmonella spp., Shigella spp., and Campylobacter spp. Moreover, KOH treatment of feces would eliminate the need for clinical laboratories to stock one of the various selective media that have been designed to facilitate isolation of Y. enterocolitica from stool samples. These media, including CIN (10), Y (11), pectin (3), and CAL agars, are sometimes difficult to prepare and have short shelf lives. In contrast, most laboratories already stock KOH pellets and Mac agar.

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LITERATURE CITED


