Identification of *Yersinia* Species by the API 20E

NARINDER K. SHARMA,1 PATRICK W. DOYLE,1* SHARON A. GERBASI,1 AND JOHN H. JESSOP2

McNair Clinical Laboratories, 201-605 West 8th Avenue, Vancouver, British Columbia V5Z 1C7,1 and British Columbia Centre for Disease Control, Vancouver, British Columbia V6J 4M3,2 Canada

Received 27 December 1989/Accepted 20 February 1990

A prospective study was performed to assess the effectiveness of the API 20E in the identification of 183 *Yersinia* isolates incubated at 28°C for 18 to 24 h. The results showed an overall correct-identification rate of 90%, with positive predictive values for *Yersinia enterocolitica* and *Yersinia frederiksenii* of 94 and 92%, respectively. *Yersinia intermedia* results were unacceptable.

*Yersinia enterocolitica* has been recognized as a cause of acute and chronic gastroenteritis (9). In a recent study, performed in the area of Vancouver, British Columbia, Canada, *Yersinia* spp. were isolated from 11.4% of patients examined (5). The species to which *Yersinia* isolates belong should be identified to help assess their pathogenic significance, since *Y. frederiksenii, Y. intermedia,* and *Y. kristensenii* are considered less pathogenic than *Y. enterocolitica* and *Y. pseudotuberculosis* (2, 7). There is a paucity of information in the literature on the effectiveness of the API 20E (Analytab Products, Plainview, N.Y.) for the identification of *Yersinia* species (1). This study was performed to prospectively assess the API 20E, a microtube biochemical identification system for members of the family *Enterobacteriaceae* and other gram-negative organisms, for its ability to identify *Yersinia* species.

(This work was presented in part at the 56th Conjoint Meeting on Infectious Diseases, Calgary, Alberta, Canada, 20 to 24 November 1988.)

We analyzed 175 clinical isolates of *Yersinia* spp. recovered from outpatient stool specimens over a 19-month period from 1986 to 1988 at McNair Clinical Laboratories. We also analyzed six clinical isolates of *Y. kristensenii* and two isolates of *Y. pseudotuberculosis* recovered in 1988 and 1989. Stool specimens were transported for bacterial culture in a sterile container with no fixative at room temperature. All specimens were cultured within 6 h of receipt in our laboratory.

Stool cultures were examined for all routine enteric pathogens. Cultures for *Yersinia* spp. were set up directly on *Yersinia*-Selective Agar with cefsulodin-Irgasan-novobiocin supplement (CIN) for 48 h of incubation at 28°C and also on phosphate-buffered saline for cold enrichment at 4°C (6). (Yersinia-Selective Agar, CIN, and phosphate-buffered saline were purchased from Oxoid Ltd., London, England. Media were made and quality was controlled in-house.) After 10 days of cold enrichment, the specimens were subcultured on CIN at 28°C for 48 h.

All organisms growing on the CIN plate were subcultured on a Simmons citrate agar slant (Difco Laboratories, Detroit, Mich.) and a Mueller-Hinton plate (Prepared Media Laboratories, Richmond, British Columbia, Canada). An oxidase test was performed on the Mueller-Hinton plate. All citrate-negative, oxidase-negative organisms were set up on an API 20E as suspected *Yersinia* spp. Citrate-positive organisms and oxidase-positive organisms were not evaluated further for this study. Farmer et al. have found that 15% of *Y. frederiksenii* isolates and 5% of *Y. intermedia* isolates are citrate positive, and such isolates would have been missed by our citrate screening (4). In view of the less-pathogenic nature of these organisms, we feel this screening method was acceptable. Isolates for identification by the API 20E were picked from the Mueller-Hinton plate. API strips were inoculated according to the recommendations of the manufacturer, with the exception of incubation at 28°C. Data from Archer et al. have shown better identification with the API 20E at 28°C than at 37°C (1). API strips were read at 18 to 24 h.

All organisms were referred to the British Columbia Centre for Disease Control for definitive identification. Reference testing was performed by standard tubed biochemical tests and the methods of Ewing (3). Isolates incorrectly identified at McNair Clinical Laboratories were retested with an API 20E strip, using a 48-h incubation period at 28°C, to see whether isolates misidentified at 25 h could be correctly identified at 48 h of incubation.

Data were collected and analyzed with a personal computer with the Symphony software program (Lotus Development Corp., Cambridge, Mass.). The sensitivity, specificity, positive and negative predictive values, and prevalence were determined by using the reference result (8). The identities of patients were kept confidential.

Over the period of study, 183 *Yersinia* isolates were identified to the species level. Our overall correct identification rate was 90% (Table 1). For the API code numbers 1154733, 1154773, and 1155773, multiple errors were made. We examined individual biochemical tests to see where the problems were arising in identification. Errors which affected identification of species were made only in three tests: inositol, rhamnose, and melibiose. In assessing the significance of these errors, we found that the positive predictive values for inositol and rhamnose were acceptable at 99 and 94%, but melibiose had a positive predictive value of 10%. Fifty percent of the errors for melibiose could be corrected with repeat testing with a 48-h incubation, and most of these errors involved *Y. intermedia*. Special care should be taken in the reading of these three sugars, and the instructions of the manufacturer should be followed. Archer et al. found that the inability to ferment melibiose and rhamnose at 37°C resulted in most of their incorrect identifications (1). Punsland et al. found delayed utilization of rhamnose, melibiose, and citrate to be a problem in identification of *Y. intermedia* (7).

In a similar study of 105 isolates of *Yersinia* spp., Archer et al. found overall identification rates of 51% at 37°C, 66%...
TABLE 1. Yersinia identification by API 20E

<table>
<thead>
<tr>
<th>Reference identification organism</th>
<th>No. of isolates</th>
<th>No. of correct identifications (% of total)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. enterocolitica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>116 (97)</td>
<td>97</td>
<td>88</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Biotypes 3, 4, and 5</td>
<td>31</td>
<td>31 (100)</td>
<td>100</td>
<td>99</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Biotypes 1 and 2</td>
<td>88</td>
<td>85 (97)</td>
<td>97</td>
<td>94</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Y. frederiksenii</td>
<td>46</td>
<td>36 (78)</td>
<td>78</td>
<td>98</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Y. intermedia</td>
<td>6</td>
<td>1 (17)</td>
<td>17</td>
<td>95</td>
<td>11</td>
<td>97</td>
</tr>
<tr>
<td>Y. kristensenii</td>
<td>9</td>
<td>9 (100)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>3</td>
<td>3 (100)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>165 (90)</td>
<td>90</td>
<td>96</td>
<td>79</td>
<td>97</td>
</tr>
</tbody>
</table>

* PPV, Positive predictive value; NPV, negative predictive value (8). at 28°C, and 93% at 28°C if a Voges-Proskauer test was recorded as negative (1). Voges-Proskauer test results did not affect our results. Unlike Archer et al., we used the recommendations of the manufacturer in preparing the inoculum.

Unlike that reported previously by Archer (1), our identification of Y. enterocolitica biotypes 1 and 2 (indole positive) and biotypes 3, 4, and 5 (indole negative) was excellent, with positive predictive values of 94 and 99%, respectively (Table 1). No problems were encountered with the identification of Y. kristensenii or Y. pseudotuberculosis.

In conclusion, we feel that Y. enterocolitica, Y. frederiksenii, and Y. kristensenii can be identified with confidence to the species level with the API 20E incubated at 28°C and read at 18 to 24 h. We suggest repeat testing with incubation for 48 h for all other Yersinia spp. If, on repeat testing, an isolate is found to be Y. enterocolitica, Y. frederiksenii, or Y. kristensenii, then it is reported as such. If it is any other species, we recommend referral to a reference laboratory for identification. This approach would have corrected 5 of 18 misidentifications in our study, for an overall correct identification rate of 93%. We are currently employing this method in our laboratory.

We thank the staff at McNair Clinical Laboratories and M. Noble for helping us produce this research paper.

LITERATURE CITED