Simplified Scheme for Identification of Prompt Lactose-Fermenting Members of the Enterobacteriaceae

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A brief, simplified scheme involving the spot indole test and colonial morphology was evaluated for genus level identification of prompt lactose-fermenting (PLF) members of the Enterobacteriaceae. One hundred and ninety-four consecutive, clinically important PLF gram-negative rods isolated in a clinical microbiology laboratory were identified by this simplified scheme, as well as by standard biochemical tests, and the API 20E (Analytab Products, Inc., Plainview, N.Y.) system. In the simplified scheme a flat, spot indole-negative colony was identified as Escherichia coli. Spot indole-negative organisms forming mucoid colonies were identified as Klebsiella sp. or Enterobacter sp. on the basis of semisolid motility and ornithine decarboxylase tests. Approximately 94% of the study isolates followed reactions typical for E. coli, Klebsiella sp., and Enterobacter sp. as defined by this simplified scheme. When compared with the standard and Analytab Products Inc. identifications, the overall accuracy was 97.4%. The accuracy of identification of E. coli, Klebsiella sp., and Enterobacter sp. was 98.1%, 95.6%, and 87.5%, respectively. This simplified scheme is recommended for identification of selected PLF isolates in the clinical microbiology laboratory.

Many rapid techniques (2, 15, 17, 19), biochemical test schemes (4, 7, 9, 10, 12–14, 18, 21, 24), and miniaturized methods (8, 11, 16, 20, 18, 23) have been devised for identification of the Enterobacteriaceae. Recommended initial testing for members of the Enterobacteriaceae varies from three (24) to a battery of twenty or more (20, 23) tests. We have found the biochemical battery approach useful for identification of nonlactose-fermenting gram-negative rods, but question the need for a battery of tests for all prompt lactose-fermenting gram-negative rods since the taxonomic probabilities are comparatively restricted. The purpose of this paper is to evaluate the accuracy of a diagnostic scheme which allows the identification of Escherichia coli isolates with typical colonial morphology using only a spot indole test (22) and Klebsiella-Enterobacter isolates with colonial morphology typical for that group with two additional tests.

MATERIALS AND METHODS

Bacteria. One hundred and ninety-four isolates of prompt lactose-fermenting gram-negative rods consecutively submitted for antimicrobial susceptibility testing in the clinical microbiology laboratory of the Arizona Medical Center were included in the study. For the purposes of the study, a prompt lactose fermenter (PLF) was defined as any gram-negative rod demonstrating a pink-to-red colony on MacConkey agar (Difco Laboratories, Detroit, Mich.) after overnight incubation at 35°C. Cultures with mixtures of gram-negative rods were subcultured before applying diagnostic criteria. Blood culture isolates were excluded because more extensive testing is routinely performed. The organisms were tested immediately or maintained on nutrient agar slants at room temperature before testing.

Identification procedures. During the study period, technologists in the clinical laboratory followed the simplified identification scheme outlined in Fig. 1 for PLF isolates. Initial classification was based on correlation of the colonial morphology on MacConkey agar with the spot indole reaction. Raised mucoid colonies characteristic of the Klebsiella-Enterobacter group were designated "mucoid" and all others were called "flat" without regard to other features of colonial structure. A flat PLF with a positive spot indole was identified as E. coli. A mucoid PLF with a negative spot indole was considered a member of the Klebsiella-Enterobacter group and identified to genus level with semisolid motility (1) and ornithine decarboxylase tests. If the organism was nonmotile and ornithine negative, it was identified as Klebsiella. If the organism was motile and the ornithine positive, it was identified as Enterobacter. All other patterns were considered atypical, and a 12-test biochemical battery similar to that outlined below was inoculated for identification using the tables of Edwards and Ewing (5). The technologists were allowed to follow the preexisting
practice of using antibiotic susceptibility patterns as a clue to misidentification during the study period. For example, a flat indole-positive PLF with a susceptibility pattern typical of Klebsiella would receive more extensive testing.

The spot indole test was performed as described by Vracko and Sherris (22). Briefly, a loopful of a single, well-isolated colony from the primary blood agar plate was rubbed onto filter paper saturated with a 5% solution of p-dimethylaminobenzaldehyde in 10% aqueous HCL. An indole-positive colony turns pink to red within 2 to 5 s, and an indole-negative colony gives no color change. The saturated filter paper was maintained in a petri dish and used to test many different isolates during the following 2 to 3 h.

All isolates initially identified by the simplified scheme in the clinical laboratory were independently identified using the procedures and diagnostic tables of Edwards and Ewing (5). The tests used were indole, Moeller ornithine and lysine decarboxylase and arginine dihydrolase, triple sugar iron, Christensens urea, lysine iron agar, Simmons citrate, nitrate reduction, methyl red, Voges-Proskauer, semisolid motility (1), and acid and gas production from 1% dextrose, lactose, sucrose, and mannitol in fermentation base. Media were prepared from Difco dehydrated reagents. In addition, all study isolates were tested with the API 20E multipurpose micromethod system (Analytab Products, Inc., Plainview, N.Y.) according to manufacturer’s instructions for inoculation and identification using the API code and profile register.

If the Edwards and Ewing and the API identifications agreed, the result was accepted as correct without further testing. Disagreements were resolved by repeat or further testing with the Edwards and Ewing result defined as correct in the single instance where the two biochemical batterys did not agree.

RESULTS

Of the 194 PLF strains studied, 163 (84%) were E. coli, 23 were Klebsiella, and 8 were Enterobacter. All Klebsiella isolates were K. pneumoniae, and of the 8 Enterobacter isolates, 5 were E. cloacae, and 3 were E. aerogenes. One hundred and eighty-two (93.8%) of these followed the reactions shown in Fig. 1 for typical E. coli, Klebsiella, or Enterobacter and were identified as such in the simplified scheme without additional biochemical testing. All but three of these identifications were correct. The remaining 12 strains were atypical as defined in Fig. 1, and additional biochemical tests were used for identification.

The sources of the organisms tested and the overall accuracy of the simplified scheme are shown in Table 1. Most E. coli isolates were from urine (128 out of 163). Klebsiella isolates were evenly distributed between urine (11 out of 23) and nonurinary (12 out of 23) sites and 6 of 8 Enterobacter isolates were from nonurinary sources.

The overall accuracy of the simplified identification scheme was 97.4% (189 out of 194) with the accuracy of identification of E. coli, Klebsiella, and Enterobacter isolates being 98.1% (160 out of 163), 95.6% (22 out of 23), and 87.5% (7 out of 8), respectively (Table 1). E. coli was accurately identified 98.4% (126 out of 128) of the time from urinary sources and 97.1% (34 out of 35) of the time from nonurinary sources. Identification accuracy for Klebsiella isolates was 100% (11 out of 11) and 91.7% (11 out of 12) from urinary and nonurinary sources, respectively. There were two Enterobacter isolates from the urine (both E. aerogenes). One was identified correctly. The remaining six Enterobacter isolates from nonurinary sources were identified correctly.

A detailed analysis of the five incorrect identifications is shown in Table 2. The first two were due to failure to follow the test protocol. The first of these was a flat indole-positive E.

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**Table 1. Accuracy of a simplified scheme for identification of prompt lactose-fermenting gram-negative rods**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>Identification*</th>
<th>No.</th>
<th>No. (%) correctly identified by simplified scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Urinary</td>
<td>128</td>
<td>128</td>
<td>(96.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella</td>
<td>11</td>
<td>11 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>138</td>
<td>(97.8)</td>
</tr>
<tr>
<td>E. coli</td>
<td>Nonurinary</td>
<td>35</td>
<td>34</td>
<td>(97.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella</td>
<td>12</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter</td>
<td>6</td>
<td>6 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>(96.2)</td>
</tr>
<tr>
<td>E. coli</td>
<td>All sources</td>
<td>163</td>
<td>160</td>
<td>(98.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella</td>
<td>23</td>
<td>22 (95.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter</td>
<td>8</td>
<td>7 (87.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>189</td>
<td>(97.4)</td>
</tr>
</tbody>
</table>

* As determined by biochemical methods of Edwards and Ewing (5).
Klebsiella-Enterobacter coli isolates in clinical of the multitestported here of the sequential isolates from of and Enterobacteriaceae.

The overall 97.4% genus level accuracy of this scheme is comparable to the 98.8%, 96.9%, 96.4%, and 87.4% reported for the R/B system (18), API 20E (16), Enterotube (21), and MiniTek (18), respectively. The surprising accuracy of this brief scheme should be interpreted in view of the select population studied here, i.e., sequential isolates from a clinical microbiology laboratory. Therefore, the accuracy figures reported here are not entirely analogous to those of the multistep systems cited above in which clinical isolates and stock organisms were included in order to obtain a broader representation of organisms. Although the latter systems are applicable to a larger group of organisms, the overall accuracy of identification of E. coli by this brief scheme is 98.1% (98.4% from urine) which is comparable to the 92.9, 96.4, 93.3, and 100% reported by API 20E (20), Enterotube (21), PathoTec (19), and Auxotab (15) methods, respectively. The overall accuracy of Klebsiella identification in the present paper is 96.6% which is also comparable to 93.3, 100, 100, and 80 to 100% reported for Auxotab (15), Enterotube (21), API 20E (20), and PathoTec (19), respectively. Others have reported identification methods for PLF isolates using three (24) or four (4) tests requiring overnight incubation with good correlation with conventional methods.

Although not encountered in this study, a number of organisms could be misidentified using the scheme described here. Citrobacter diversus could be misidentified as E. coli. Approximately 9% (5) of Aeromonas species are prompt lactose fermenting and may produce a colony similar to E. coli. Arizona species frequently (61.3%) ferment lactose and a rare (2%) isolate is indole positive (5). The nonpigmented, indole-positive Enterobacter agglomerans may be misidentified as E. coli. Rare lactose-fermenting Serratia rubidaea and members of the Proteus-Providence group may be misidentified, but in most instances would give atypical patterns. (Atypical isolates of the more common PLF Enterobacteriaceae such as indole-positive Klebsiella or indole-negative E. coli could also pose an identification problem, but in most cases these strains would give atypical reaction patterns [Fig. 1] since the colonial morphology must fit the indole reaction. Although Enterobacter agglomerans, Serratia rubidaea and Providencia species are not recognized in Bergey's Manual of Determinative Bacteriology, 8th ed. [5], they are in common usage today.) Strain 5 (Table 2) was the only one of four indole-positive Klebsiella isolates encountered in the present study which was misidentified, but the error was due to atypicality of both the indole and colonial morphology. Although colonial morphology is admittedly subjective, it

<table>
<thead>
<tr>
<th>Correct identification*</th>
<th>Brief scheme identification</th>
<th>Spot indole</th>
<th>Colonial morphology</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Klebsiella</td>
<td>+</td>
<td>Flat</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Klebsiella</td>
<td>+</td>
<td>Mucoid</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Klebsiella</td>
<td>-</td>
<td>Unspecified</td>
<td></td>
</tr>
<tr>
<td>Enterobacter</td>
<td>E. coli</td>
<td>+</td>
<td>Flat</td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>E. coli</td>
<td>+</td>
<td>Flat</td>
<td></td>
</tr>
</tbody>
</table>

* As determined by biochemical methods of Edwards and Ewing (5).
can be sufficiently characteristic to indicate genus level identity (6). Although no one would recommend using colonial morphology alone to identify PLF isolates, experience in this study indicates that, when combined with simple objective tests, it can be consistently useful.

Two of the misidentifications listed in Table 2 resulted from failure of the spot indole to correlate with the tube test. False-negative spot indole tests rarely occur (22), and a false positive may reflect indole production by an adjacent indole-positive colony which is not adequately isolated from the colony in question. The present study confirms the good correlation between tube and spot indole tests. The remaining two instances of incorrect organism identification resulted from human error. The specified identification procedures were not followed.

This study shows that the combination of the spot indole test and colonial morphology, plus two biochemical tests in some instances, gives an identification of PLF gram-negative rods with an accuracy acceptable for many significant laboratory isolates. This brief scheme is, therefore, recommended for identification of PLF isolated from sites where a small degree of error can be tolerated, such as urine. Isolates from blood cultures and CSF should routinely receive more extensive testing.

ACKNOWLEDGMENT

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LITERATURE CITED