Four-Hour Urease Test for Distinguishing Between Klebsiella and Enterobacter

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Infections with Klebsiella and Enterobacter have increased among hospitalized patients. To study such infections, relatively simple but precise methods are needed for clinical laboratories to identify the two genera accurately. Moreover, a rapid identification is essential for assisting with the therapy of the patients. For this purpose, a new 4-hr urease test was developed so that colonies could be tested directly from blood-agar plates which have been inoculated with clinical material and allowed to incubate overnight. This 4-hr test was positive with 98.5% of 202 Klebsiella species and negative with 80 Enterobacter species. As a single criterion for distinguishing between the two major genera, the new 4-hr urease test was just as accurate as a motility test (99% of the 282 isolates were accurately identified with either). The 4-hr urease test represents a simple, rapid, and reliable technique which is ideally suited for use in clinical microbiology laboratories.

Klebsiella and Enterobacter have been increasing in prevalence as the cause of serious nosocomial infections (4, 12, 14). The appearance of strains which are resistant to multiple chemotherapeutic agents has created serious problems in the treatment of such infections and has emphasized the importance of controlling the spread of microorganisms throughout the hospital (11). Other investigators (4, 12) have described differences in the clinical and epidemiological characteristics of infections caused by Klebsiella species and those due to Enterobacter species. In addition, the two genera differ significantly in their susceptibility to various chemotherapeutic agents, especially cephalothin and related drugs (2–4, 6, 7, 9, 10). For these reasons, it is becoming increasingly important for diagnostic laboratories and for clinical investigators to distinguish accurately between the two genera.

For practical reasons, it is unlikely that the presently accepted nomenclature will be widely utilized in clinical laboratories until relatively simple, rapid methods are developed. In 1957, Hormaeche and Munilla (8) described a "quick" urea test which helped to distinguish Klebsiella (96% positive) from Enterobacter (all negative). The present report describes a similar technique which is slightly more sensitive and which permits colonies to be tested directly from blood-agar plates that have been inoculated with clinical material and allowed to incubate overnight. This new 4-hr urease test was evaluated by testing 282 Klebsiella-Enterobacter species and comparing the results with those obtained with motility and ornithine decarboxylase tests.

MATERIALS AND METHODS

The cultures used in this study were all recent clinical isolates, initially selected on the basis of rapid indole (13) and Voges-Proskauer (1) tests. All strains were then examined for ornithine and lysine decarboxylase and motility and urease activity. Other tests were carried out as needed, by using the methods of Edwards and Ewing (5). Motility was detected after 18 to 24 hr at 37 C in G-I motility medium (Difco) with 0.001% tetrazolium chloride. Prolonged incubation or utilization of lower temperatures did not alter the results of these motility tests. Moeller’s decarboxylase broth (Difco), with a sterile mineral oil seal, was used for ornithine and lysine decarboxylase tests. These tests, along with the necessary control tube, were examined daily for 4 consecutive days at 37 C. Most positive reactions appeared on the first day, but a few required 2 days and negative tubes did not change after 48 hr.

For the new 4-hr urease test, a stock urea reagent was prepared by adding 4 g of urea to 104 ml of distilled water containing 1/10,000 KHP04; 8 ml of a cresol red indicator was then added. The indicator had been prepared as a stock reagent by adding 0.1 g of cresol red and 13.1 ml of 0.02 N NaOH to 237 ml of distilled water. Sterilization was not needed, and
the reagents could be stored at 4°C for at least 3 months. The day the tests were performed, 0.2-ml portions of this urea solution were transferred aseptically to tubes (13 by 100 mm). To perform the test, colonies were selected from 18- to 24-hr blood-agar plates [Trypticase Soy Agar (BBL) with 5% defibrinated sheep blood]. A dense suspension from several colonies was prepared in the small volume of urea reagent. After 4 to 6 hr in a 50°C heating block, a pink to purple color indicated a positive reaction, whereas negative tests were yellow to white in color. If a very light suspension was tested, false-negative results were obtained, and false-positive results occurred when colonies were selected from a medium containing fermentable carbohydrates or from plates incubated longer than 24 hr.

This new 4-hr urease test was first compared with the results obtained with the "quick" test and the "slow" test of Hormaeche and Munilla (8). For both of the latter tests, the inoculum must be obtained from a 24-hr agar culture. The composition of the agar medium used to prepare the inocula was modified only in that we utilized Bacto-peptone (Difco) rather than Evans peptone. For the "quick" test, a 2% solution of urea with a cresol red indicator was heavily inoculated and allowed to incubate at 35°C for 2 hr, at which time positive tests were reddish-purple in color. For the "slow" test, a 0.2% solution of urea in peptone broth with a cresol red indicator was inoculated and observed for 4 days.

RESULTS

The new 4-hr urease test was compared with the "quick" test and the "slow" test of Hormaeche and Munilla (8), by using 103 Klebsiella-Enterobacter stock cultures (Table 1). Hormaeche and Munilla (8) observed more strains of Klebsiella to be urease-positive with their 2-hr "quick" test than with their 4-day "slow" test, but, in the present series, 66 of the 70 Klebsiella species were positive with the "slow" test and only 62 were positive with the "quick" test (Table 1). In comparison, the new 4-hr urease test was positive with all but one of the 70 Klebsiella species. All 33 strains of Enterobacter were negative by all three tests.

The new 4-hr urease test was then evaluated more extensively by testing 282 recent clinical isolates, including 202 Klebsiella species, 52 E. cloacae, 26 E. aerogenes, and 2 E. liquifaciens (Table 2). Positive tests were obtained with 98.5% of the Klebsiella species, and the Enterobacter species were all negative. Three Klebsiella isolates (1.5%) were negative with the 4-hr urease test and would have been reported to be Enterobacter if further testing had not been performed (all were nonmotile and ornithine decarboxylase negative). On the other hand, 4% of the Enterobacter strains (two E. aerogenes and one E. cloacae) were initially nonmotile and would have represented exceptions in the other

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of strains</th>
<th>Results of urea tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow*</td>
<td>Quick*</td>
<td>4-Hr*</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>62</td>
<td>+         +</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>4</td>
<td>+         -</td>
</tr>
<tr>
<td>E. liquifaciens</td>
<td>3</td>
<td>-         -</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>33</td>
<td>-         -</td>
</tr>
</tbody>
</table>

* Methods of Hormaeche and Munilla (8).

** Table 1. Comparison of urea tests with 70 Klebsiella and 33 Enterobacter species.**

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of strains</th>
<th>Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Orn*  Motb  Urea*</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>199</td>
<td>-      -     +</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>76</td>
<td>+      -     -</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>3</td>
<td>+      -     -</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>4</td>
<td>-      -     -</td>
</tr>
<tr>
<td>E. liquifaciens</td>
<td>1</td>
<td>-      -     -</td>
</tr>
</tbody>
</table>

* Ornithine decarboxylase (1% in Moeller's base) read daily for 4 days at 37°C.

** Table 2. Characteristics of 282 clinical isolates of Klebsiella-Enterobacter.**

**DISCUSSION**

In clinical microbiology, it is neither possible nor necessary to test all bacterial isolates with a large series of biochemical tests, many of which require prolonged periods of incubation. Instead, a few well-selected rapid tests can provide accurate identification of all but a few strains with exceptional characteristics. We recently reported a practical system for the rapid identification of
the more common prompt lactose-fermenting strains of Escherichia coli, Klebsiella, and Enterobacter (2). This identification scheme utilizes a rapid indole test (13), a rapid Voges-Proskauer test (1), and the 4-hr urease test (described in this report). These three rapid procedures are practical tests for quickly identifying the more common lactose-positive gram-negative bacilli.

Klebsiella species are usually distinguished from Enterobacter species by motility or ornithine decarboxylase, or both. The 282 isolates included in the present report would have been identified accurately provided both motility and decarboxylase tests were performed. With both tests, an accurate identification would have been obtained after 18 to 24 hr of incubation, since the Klebsiella species were negative with both tests and all of the Enterobacter species were positive in at least one of the two tests after overnight incubation. However, if the sole criterion had been the ornithine decarboxylase tests read only after overnight incubation, five Enterobacter species (6%) would have been erroneously identified as Klebsiella. Four of these five strains turned positive after 2 days of incubation. On the other hand, if motility had been the only criterion, all but three of the isolates would have been identified accurately. The motility medium used in this study provided a satisfactory test but required overnight incubation. Motility could have been detected more quickly with a wet-mount preparation. However, this technique is not entirely satisfactory, in the hands of technologists inexperienced with its technical pitfalls. In addition, routine use of the wet-mount preparation for motility tests with large numbers of cultures is impractical because of the time required to prepare and examine the preparations. The 4-hr urease test is not only simpler to perform and easier to read but is equally as accurate as a motility test for distinguishing between the two genera. In this series, 99% of the isolates were accurately identified with either the 4-hr urease test or the motility test which required an overnight incubation.

In this report, all urease tests were performed with colonies selected from Trypticase Soy Agar with 5% defibrinated sheep blood. Preliminary studies indicate that slightly different results may be obtained if other agar media are used. On media containing fermentable carbohydrates, Enterobacter species gave positive reactions, and cultures incubated longer than 24 hr also tended to produce false-positive tests. An extremely turbid suspension must be used to avoid false-negative results. The total volume of test solution was reduced so that an adequately dense suspension could usually be obtained with two or three isolated colonies.

This test is optimal for use in clinical microbiology laboratories where time, as well as accuracy, should be of major importance. The technique is rapid, simple to perform, easy to read, and utilizes inexpensive, stable reagents. For these reasons, the 4-hr urease test is recommended as a routine procedure for distinguishing between the genera Klebsiella and Enterobacter.

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LITERATURE CITED