Ten-Minute Test for Differentiating Between
Klebsiella and Enterobacter Isolates

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A 10-minute test, utilizing a urease paper-reagent strip (PATHO-TEC), for differentiating Klebsiella and Enterobacter species is described. By using a heavy suspension of organisms and 50 C temperature for incubation, 93% of Klebsiella strains (186/200) were positive and 95% of Enterobacter strains (190/200) were negative with this testing system. The rapid nature of the test (10 min), the facility with which it can be carried out, and the ease with which the strips can be stored and handled may make this a useful aid for the clinical microbiologist.

At the University of Minnesota Hospitals, serious infections due to the Klebsiella-Enterobacter group of organisms have more than doubled during the past 10 years, in parallel with other university centers (4, 12, 13). Furthermore, the pattern of isolation of these two genera differs with relation to clinical infection. Data from our Diagnostic Microbiology Laboratory indicates that Klebsiella is isolated approximately three times more frequently in cases of bacteremia than is Enterobacter, and, similarly, averages over the past 8 years show that Klebsiella strains comprise 18.7% of all urinary tract bacterial isolates, whereas Enterobacter makes up only 3.0% of organisms grown in urine specimens. In addition, our statistics are similar to literature reports (3, 6, 7, 9; J. A. Puopard, L. Dewees, and H. A. Morton, Proc. 9th Interscience Conf. Antimicrob. Agents Chemother., 1969) in demonstrating that these two genera manifest some differences in susceptibility patterns to certain antibiotic agents.

For these and other reasons, it seems most advisable to distinguish between Klebsiella and Enterobacter in the clinical laboratory. This paper reports the results of a paper-reagent urease strip test (furnished through the courtesy of General Diagnostics Division, Warner-Chilcott Division, Warner-Lambert Pharmaceutical Co., Morris Plains, N.J.) in accomplishing the desired differentiation.

MATERIALS AND METHODS

Two hundred isolates of Klebsiella and 200 strains of Enterobacter, including recent clinical isolates and some strains which had been stored on agar slants, were initially examined by means of Gram smear and colonial morphology. Lactose fermentation, Triple Sugar Iron agar (TSI; BBL) reaction pattern, indole production, citrate utilization, and acetyl-methyl carbinol production (Voges-Proskauer test) were assessed by using the standard methods as described by Edwards and Ewing (5). After thus determining those strains which were of the Klebsiella-Enterobacter group, testing for ornithine decarboxylase, urease production, and for motility was carried out. Ornithine decarboxylase testing was done by using decarboxylase medium base (Difco) with 0.5% of the amino acid added. These tubes were overlaid with melted paraffin after inoculation and were incubated for 24 hr at 37 C. Control broths without the amino acid were also used. Urea slants were made by the technique outlined in Edwards and Ewing (5), using urea agar concentrate (Difco). Tests were incubated at 37 C for 4 days. Motility was determined by using semisolid medium containing nutrient broth (Difco), 0.5%, 2.3-5-triphenyl-tetrazolium, and 0.5% agar (Difco), and also on S I M medium (BBL). Motility tests were incubated at 37 C for 2 days. Those cultures appearing nonmotile were held at 25 C for 5 days longer before recording them as negative.

Organisms grown overnight on TSI agar slants were used for paper-strip testing. A heavy suspension of separate test strains was made in 0.2 ml of sterile saline in culture tubes (10 by 75 mm). The urease test strips were placed in the suspension, and the tubes were incubated in a water bath at 50 C. Initial readings were made at 10 min, and all negative tests were again read at 30 min and 1 hr.

RESULTS

Organisms classified as Klebsiella were gram-negative bacilli which were lactose-positive, citrate-positive, Voges-Proskauer-positive, ornithine decarboxylase-negative, and nonmotile. Strains defined as Enterobacter were gram-negative rods which were lactose-positive, citrate-
positive, Voges-Proskauer-positive, and 194 of 200 were ornithine decarboxylase-positive, and 196 of 200 strains were motile. All Enterobacter strains were either ornithine-decarboxylase positive, or motile, or both.

By urease paper-strip reagent method, 186 of 200 Klebsiella strains (93%) tested by using inocula from TSI agar slants were positive within 10 min. Three of those strains which were negative at 10 min became positive at 30 min. In contrast, only 10 of 200 Enterobacter (5%) were positive for urease activity, when inoculum was taken from TSI slants. Of the 10 positive Enterobacter, 5 were motile, ornithine decarboxylase-negative strains, and one was a non-motile, ornithine-positive strain.

When the inoculum was taken from blood-agar plates, or when incubation was carried out at 37 C, the results with Klebsiella were somewhat poor even after 4 hr. It was apparent that the results were not as meaningful as the method using inoculum from a TSI slant and incubating at 50 C. Of the 200 Klebsiella strains tested from the TSI agar, the results with the paper-reagent strip correlated with the reading of urease activity on the urea slant in all but 6 instances. All Enterobacter strains taken from TSI slants gave results which correlated with urea-agar slant testing.

**DISCUSSION**

As with any new test to be used in the diagnostic laboratory for organism differentiation, it is hoped that this differentiation could be carried out both expeditiously and accurately. The current method used in our laboratory, and by many laboratories which do make a differentiation between Klebsiella and Enterobacter, is to employ motility or ornithine decarboxylase tests, or both, to achieve this separation once an organism has been shown to be of the Klebsiella-Enterobacter group (10). Both of these require overnight incubation and are somewhat expensive, and both tests are necessary to obtain the optimum accuracy.

Hormaeche and Munilla (8) have described a rapid test employing urea, and Barry, Bernsohn, and Thrupp (1, 2) have also described a rapid urease test for separating Klebsiella and Enterobacter. These tests require approximately 4 hr for completion. The latter authors demonstrated with their 4-hr technique that 98% of Klebsiella were positive and all Enterobacter strains were negative. Their test uses inocula from blood-agar and can thus be done from the original isolation plate in many instances. They relate that slightly different results are obtained when inoculum from other media, such as TSI, is employed.

The results obtained in the present study, by using the paper-reagent urease strips, are encouraging, in that a simple test, with 94% overall accuracy, is available for making a 10-min differentiation between Klebsiella and Enterobacter strains. The results with inocula from blood-agar were very poor, and, thus, the test as described requires growth on TSI. This fact somewhat limits the overall usefulness of the paper strip test, although for those laboratories routinely using TSI slants in their identification scheme, this presents no problem. For those laboratories routinely using the paper-reagent strips (11), this test increases the number of identifications which can be made using the PATHO-TEC strips. For clinical laboratory purposes, when one considers the amount of time saved and the facility of using paper-reagent strips, this method may be of value, and, indeed, has been in many instances in our laboratory. Laboratories not prepared to use the more involved techniques employing ornithine decarboxylase and motility media, or other schema, can separate Enterobacter from Klebsiella with reasonable accuracy by using this 10-min paper-strip urease method.

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