Rapid Method for Presumptive Identification of Corynebacterium jeikeium

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Corynebacterium jeikeium causes systemic infections, particularly in immunocompromised hosts. A minitube assay has been developed for the presumptive identification of C. jeikeium. With our rapid sucrose-urea test and conventional biochemical tests, sixty isolates of gram-positive, catalase-positive bacilli were identified in our laboratory. Results indicated that our assay has a sensitivity of 100% and a specificity of 90%.

Corynebacterium jeikeium has been well documented as the causative agent of a number of serious infections, including sepsis, prosthetic valve endocarditis, meningitis, and peritonitis, predominantly, although not exclusively, in immunosuppressed patients (2, 6). Skin colonization or superficial infections with this organism are therefore a significant clinical concern (5), and expedient differentiation of this organism from morphologically similar commensal diphtheroids is highly desirable. A number of tests have been proposed for differentiating C. jeikeium from other pleomorphic, catalase-positive, gram-positive rods. Simple phenotypic characteristics, particularly enhanced growth on lipid-containing media and resistance to multiple antibiotics, have been widely reported as having utility in the identification of this organism (7). Unfortunately, several less pathogenic diphtheroids are lipophilic, and these organisms may also be antibiotic resistant (1). In addition, not all C. jeikeium isolates demonstrate the "characteristic" antibiotic resistance pattern; a small but significant percentage (in our laboratory, approximately 10%) of isolates appear to be comparatively susceptible to antibiotics, and since the relative pathogenicity of resistant versus susceptible isolates is unknown, the use of semiselective antibiotic-containing media for the isolation of C. jeikeium (10) is not advisable. A number of rapid biochemical test systems appear to be capable of accurately identifying C. jeikeium; one of these, the API 20S (Analytab Products, Plainview, N.Y.), is used in our laboratory for the identification of C. jeikeium and CDC group D-2 (3). The principal disadvantage of many of these systems is that large inocula are required and, in our experience, much time, as well as expensive strips, can be wasted in working with ultimately insignificant isolates. We developed a minitube assay that enables two key biochemical tests, those for sucrose utilization and urease production (4), to be carried out expeditiously and with minimal inocula, providing a presumptive identification of C. jeikeium or non-C. jeikeium in 3 h or less.

Our routine bacteriology laboratory uses microscopic and macroscopic morphologies to separate the aerobic, catalase-positive, gram-positive coryneform organisms into two groups: the C. jeikeium like (coccobacilli or short rods; small shiny or gray colonies) and the non-C. jeikeium like (large or pleomorphic diphtheroidal rods or "club forms"; large opaque or white colonies). During the course of this study, all organisms isolated from clinical sources and preliminarily identified as C. jeikeium like were subjected to the rapid sucrose-urea (RSU) test. These isolates were subsequently identified to the species level with the API 20S system (3) and conventional biochemical tests. To perform the RSU test, 3 drops of RSU test medium (nutrient broth containing, per liter, 7 g of sucrose, 2 g of urea, 1 g of peptone, 0.4 ml of Tween 80, 44 mg of bromothymol blue, and 25 mg of phenol red) and 3 drops of RSU control medium (RSU test medium without sucrose and urea) were dispensed into two test tubes (6 by 50 mm). A visible inoculum of the organism (obtained from a non-glucose-containing medium) was added, and the tubes were incubated at 35°C. We found that using control medium without sucrose and urea enhanced the ability of technologists unfamiliar with the RSU test to differentiate a true sucrose-positive reaction from a slight acidification of the medium produced by a large inoculum. A positive sucrose utilization test was indicated by a yellow color, and a positive urease production test was indicated by a purple color. All isolates that produced no color change after 3 h of incubation (sucrose negative and urease negative) were presumptively identified as C. jeikeium. Color changes occurring at or before 3 h were used to rule out C. jeikeium.

The RSU medium formulation was originally designed to facilitate both the growth of diphtheroids and the colorimetric detection of products of metabolism. Initial experiments demonstrated, however, that even with small inocula (two or

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>No. of isolates that were:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose positive</td>
</tr>
<tr>
<td>C. jeikeium (30)</td>
<td>0</td>
</tr>
<tr>
<td>CDC group A (5)</td>
<td>5</td>
</tr>
<tr>
<td>CDC group D-2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>CDC group F-2 (1)</td>
<td>0</td>
</tr>
<tr>
<td>CDC group G-1 (2)</td>
<td>2</td>
</tr>
<tr>
<td>CDC group G-2 (16)</td>
<td>15</td>
</tr>
<tr>
<td>C. minutissimum (1)</td>
<td>1</td>
</tr>
<tr>
<td>Other coryneforms (2)*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Organisms that we were not able to identify to the species level in our laboratory.
three colonies), growth was not necessary to enable a detectable change in the pH of the medium if the organism was capable of utilizing sucrose or deaminating urea. Attempts to replace the nutritional components of the medium with buffers commonly available in the clinical laboratory (for example, phosphate or Tris) were unsuccessful; these buffers failed to provide the pH-buffering stability necessary to permit the detection of both acidic and alkaline metabolic end products in a single medium.

The identities of the coryneform bacteria subjected to the RSU test and their RSU reactions are shown in Table 1. All 30 isolates subsequently identified as *C. jeikeium* showed a negative reaction in the RSU test. Of the 30 isolates identified as species other than *C. jeikeium*, 23 produced a yellow color in the RSU test, 4 produced a purple color (including 3 isolates of CDC group D-2), and 3 were nonreactive. On the basis of these data, the RSU test has a sensitivity of 100% and a specificity of 90%.

As can be seen from Table 1, the use of urease production as one component of the RSU test enabled this assay to function as a relatively effective screen for CDC group D-2 (100% sensitivity and 75% specificity). This organism is morphologically very similar to *C. jeikeium* and can be highly resistant to many antimicrobial agents but differs in that it appears to be primarily a pathogen of the urinary tract (8). There are published reports of serious infections with this organism. The majority of these, however, occurred in patients with underlying urinary tract disease; there is no clear evidence that skin colonization with CDC group D-2 can lead directly to a systemic infection (9). The potential clinical significance of CDC group D-2 has thus been accounted for in our algorithm. Sucrose-positive coryneforms isolated from urinary tract specimens are only examined further if, after clinical consultation, they are deemed to be potentially significant (Fig. 1). Urease-positive organisms and sucrose- and urease-negative organisms are reported as presumptively CDC group D-2 and *C. jeikeium*, respectively, inoculated into the API 20S system for identification, and submitted for susceptibility testing.

The results presented here indicate that the RSU test can be used as an efficient screening test for *C. jeikeium* and CDC group D-2. By using the RSU test in our laboratory for the algorithm shown in Fig. 1, we have effected a considerable savings in both material and time without compromising the accuracy of the information generated.

**REFERENCES**

8. Soriano, F., C. Fonte, M. Santamaria, J. M. Aguado, I.}

**FIG. 1.** Algorithm demonstrating the application of the RSU test in our routine bacteriology laboratory.
