Rapid Identification of Group JK and Other Corynebacteria with the Minitek System

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Forty primary clinical isolates and 50 stock cultures of corynebacteria and coryneform bacteria were tested with the Minitek system (BBL Microbiology Systems, Cockeysville, Md.). The Minitek correctly identified all of these organisms, including JK group isolates, within 12 to 18 h of incubation. The method does not require serum supplements for testing carbohydrate utilization by the bacteria. The Minitek system is an extremely simple and rapid way to identify the JK group, as well as many other corynebacteria, by established identification schemata for these bacteria.

Diphtheroids are generally considered normal flora in the presence of other commensal bacteria from most body sites, but there are occasions when their presence in a specimen may be considered clinically significant. Accordingly, diphtheroids have been reported in association with a variety of diseases and from many clinical sites (3, 13, 14, 17).

Corynebacteria from infected skin, soft tissues, lymph nodes, and blood or body fluids should be identified when they are derived from immunocompromised hosts (17). The need for identification of group JK corynebacteria from patients having clinical evidence of infection is now well established (23, 28). These diphtheroid or diphtheroidlike bacteria were designated group JK by the Special Bacteriology Section of the Centers for Disease Control, Atlanta, Ga. (21). An important feature of the JK group is its resistance to almost all presently available antibiotics (7, 9, 21, 27). It may be that the multiresistant isolates are mutants of susceptible resident skin flora that multiply under the selective responses of antibiotic therapy (17, 24).

Septicemia (4, 21) caused by this group of bacteria was shown to occur in patients who have predisposing illnesses, such as neoplasms (7, 10, 19, 23), ventricular shunts, or prosthetic valves (5, 10, 18, 25). JK group isolates have been isolated from a patient with an epicardial pacemaker (8). The JK group have been associated with community-acquired (4) and hospital-acquired (20, 21) infections.

Corynebacteria are most often characterized entirely by their gram stain morphology and respective growth characteristics (3). Many of the diphtheroids are relatively inactive biochemically and thus have often been difficult to characterize. A few methods have been recently proposed to differentiate the corynebacteria (1, 3, 12, 15, 21, 26).

The present investigation concerns the evaluation of the Minitek (BBL Microbiology Systems, Cockeysville, Md.) identification system as a rapid and uncomplicated way to identify the JK group of coryneforms as well as many other clinically important diphtheroids.

MATERIALS AND METHODS

Bacteria. Forty primary strains of JK group diphtheroids were originally derived from blood cultures from this institution. These were identified as JK group organisms by the criteria of Riley et al. (21). Four strains of multiply antibiotic-resistant group JK organisms were kindly supplied by R. E. Weaver and D. G. Hollis of the Special Bacteriology Section, Centers for Disease Control. All 44 JK group organisms were highly resistant to most antimicrobial agents when tested by disk diffusion. The following Corynebacterium strains were also obtained from the Centers for Disease Control: C. aquaticum (five strains), C. bovis (three strains), C. diphtheriae (two strains), C. haemolyticum (Arcanobacterium haemolyticum) (2) (two strains), C. kutscheri (one strain), C. minutissimum (two strains), C. pseudodiphtheriticum (two strains), C. renale (five strains), C. striatum (one strain), C. pseudotuberculosis (two strains), C. ulcerans (four strains), C. xerosis (five strains), group F-1 (one strain), group F-2 (one strain), group I-1 (one strain), group I-2 (one strain), group E (one strain), and group D-2 (one strain).

Stock cultures of C. diphtheriae (1 strain), and C. ulcerans (1 strain) obtained from our collection were originally maintained in Trypticase soy broth (BBL) with 10% glycerol at −70°C (22). Two strains each of C. genitalium biotype IV (ATCC 33033) and biotype II (ATCC 33031) and two strains each of C. pseudogenitalium biotype C-12 (ATCC 33035) and biotype C-2 (ATCC 33036) were obtained from the American Type Culture Collection, Rockville, Md.

All of the strains of bacteria were grown for 18 h on Columbia sheep blood agar (Scott Laboratories, Inc., Fiskeville, R.I.) with aerobic incubation at 35°C. The colonies were examined for hemolytic activity and coryneform morphology by Gram staining and tested for catalase activity and motility.

Minitek system. The Minitek system consists of paper disks impregnated with various substrates. The Minitek disks used for this investigation included: glucose, maltose, sucrose, mannitol, xylose, esculin, nitrate, urea, and o-nitrophenyl-β-D-galactopyranoside (ONPG). Minitek levulose (fructose) disks were used to differentiate C. genitalium from C. pseudogenitalium (6). A turbid suspension of each isolate (approximately a no. 5 McFarland standard) was prepared in vials of Minitek enteric and nonfermenter broth. In some instances, 1-ml vials of sterile brain heart infusion broth (BBL) prepared in this laboratory were used to prepare the respective suspensions of bacteria for inoculation onto Minitek disks.

For use, the respective disks were placed into individual wells of Minitek plastic plates. Each disk was inoculated with 2 drops of the prepared bacterial suspension by means

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of a 1-ml sterile pipette. The urea disk was overlaid with a drop of sterile mineral oil. The inoculated plates were then placed in a Minitek humidor and incubated aerobically at 35°C for 12 to 96 h. The color responses corresponding to positive and negative reactions for each of the substrate disks were interpreted as directed by the manufacturer. The nitrate disks were tested for nitrate reduction with sulfanilic acid and N,N-dimethly-l-naphthylamine reagents and for denitrification with zinc dust. The observed reactions of all inoculated disks agreed with those reported in recent publications (3, 6, 21, 26).

RESULTS AND DISCUSSION

The Minitek substrate disks used in this investigation yielded the correct biochemical profiles associated with all of the corynebacteria and coryneform bacteria (3, 6, 21, 26) examined. The color changes of the reactive Minitek substrate disks were generally quite distinct within 12 to 18 h of incubation. All of the 44 group JK organisms acidified the glucose Minitek disk within 12 to 18 h of incubation. Only 2 of the 44 strains of the JK organisms in this investigation acidified maltose by 72 h of incubation. The two C. genitalium strains acidified this carbohydrate by 72 h of incubation. Several investigators have shown that about 40% of the group JK strains ferment maltose within 3 or more days of incubation (21, 26). Furthermore, Coyle et al. (3) found that C. genitalium yields a relatively weak fermentation of maltose. Based on colony morphology, fermentation profiles, and vancomycin sensitivity, it was suggested that group JK organisms are biotypes of C. genitalium and C. pseudogenitalium (6). C. genitalium strains may be differentiated from other diphteroids by their ability to grow on blood agar but not on commercially prepared chocolate agar (3). Since only a relatively small percentage of group JK organisms may ferment maltose (21, 26), fermentation of this carbohydrate appears not to be significant for their identification. It is possible that the maltose incorporated in the Minitek disks is more pure and stable than that used in previous investigations using carbohydrate fermentation in broth medium. Accordingly, delayed or weak fermentation of maltose (3, 21, 26) may be due to glucose contamination of the maltose in the fermentation media. The results are summarized in Table 1. Both strains of C. pseudogenitalium examined in this investigation acidified fructose Minitek disks, whereas the two strains of C. genitalium did not acidify these carbohydrate disks (5). No difference in reactions occurred when the organisms were suspended in Minitek enteric, Minitek nonfermenter, or brain heart infusion broth.

Riley et al. (21) have described a method for differentiating group JK microorganisms from other corynebacteria encountered in clinical specimens. With their method, positive carbohydrate fermentation responses were generally observed by the latter and by other investigators (6, 23, 27) from 2 or more days of incubation with the respective filter-sterilized carbohydrates. Furthermore, supplementation with either rabbit or horse serum was often required for the fermentation of carbohydrates to obtain consistent reactions for group JK organisms as well as corynebacterial species (9, 17, 21, 25, 27). A rapid fermentation test was previously described for determination of carbohydrate reactions of various fastidious bacteria including the JK group and other Corynebacteria species (11). This procedure permitted identification of these organisms within 30 min to 4 h without serum supplementation. The present investigation demonstrated that serum supplementation is not required for fermentation of carbohydrates by Corynebacterium species with the Minitek system.

The API 50L (Lactobacillus) and API 20E (Enterobacteriaceae) systems have been used to classify the coryneform group (16). The latter investigation, however, did not specifically examine these two microsystems for their respective applications in the clinical bacteriology laboratory for identification of corynebacteria. Furthermore, the JK group, as well as other coryneform members, were not examined. The API 50L (Lactobacillus) strip was applied to the identification of antibiotic-resistant diphteroids (28). The
majority of the antibiotic-resistant corynebacteria strains tested with this system yielded positive reactions for glucose, galactose, and maltose. However, only 11 to 20% of the antibiotic-resistant diphtheroids were catalase negative. This finding indicates that the majority of these isolates were not members of the JK group, as the latter group were previously reported to be catalase negative (3, 21). Furthermore, a smaller group of antibiotic-resistant diphtheroids were urease positive and acidified L-arabinose and were usually methyl xyloside positive. The latter were suggested to represent a different group of antibiotic-resistant diphtheroids than those of the JK group. Furthermore, the latter investigation did not demonstrate the application of the API 50L with a relatively wide spectrum of diphtheroid species as in the present investigation.

The API 20S identification system was evaluated for the identification of antibiotic-resistant aerobic diphtheroids from blood cultures as well as from other body sites (15). For that test, the primary isolates were subcultured to yield a heavy inoculum. Once prepared, the inoculum was inoculated into the respective microwells of the API 20S strip and the reactions were read at 4 h of inoculation of the strip. The data generated from that investigation, however, did not produce sufficient information on the ability of the API 20S system to identify corynebacteria other than the JK and D2 groups (15).

Selective broth enrichment (24) and a semiselective medium (27) were previously used to detect JK group corynebacteria in clinical specimens. These media, however, do not permit differentiation of other potentially pathogenic diphtheroids.

Results of gas-liquid chromatography analysis of JK group corynebacteria and other coryneform bacteria were recently reported (1). The data did not include a wide spectrum of Corynebacterium species but indicated that the JK group was distinguishable from the other strains examined. The method, however, required a flame ionization gas chromatograph, and the test organisms were grown for 2 to 3 days under standardized growth conditions on a relatively highly enriched medium.

The present investigation is the first to show that the MiniTeck system can be used to identify a wide range of corynebacterial species, including the JK group, accurately. The method did not require the use of serum enrichments and yielded the expected characterization profiles of all of the corynebacterial microorganisms examined in this investigation. All of these organisms were clearly identifiable within 12 to 18 h of incubation of the inoculated MiniTeck system.

The MiniTeck method, as reported in this investigation, is rather simple to perform. It requires inoculation of only eight MiniTeck substrates for complete characterization in contrast to the various API systems and permits identification of Corynebacterium spp. that are at present considered of clinical importance (3). This MiniTeck method is cost effective since the use of eight substrate disks per isolate would cost approximately $1.44. The addition of a levulose (fructose) MiniTeck disk may be used to differentiate the C. pseudogenitalium biotype from C. genitalium (5).

The MiniTeck ONPG disk may be used as part of the battery of substrates to differentiate the JK group from C. luisii (3). Furthermore, as shown by Riley and associates (21), a positive ONPG response serves as a useful hallmark for the identification of other corynebacteria such as C. aquaticum and C. minutissimum. The group F-2 and I-2 isolates examined in this investigation yielded positive ONPG reactions.

This MiniTeck system provides a reproducible means of accurately identifying the JK group as well as many other corynebacteria with the application of rather easy-to-perform procedures.

Since it may be difficult as well as inaccurate to identify JK strains solely by antibiotic resistance patterns (15), the use of the MiniTeck system yields definitive identification profiles of these as well as a wide spectrum of Corynebacterium species.

The MiniTeck system, as reported herein, has been used in this clinical microbiology laboratory for over 2 years to identify antibiotic-resistant diphtheroids as well as other members of the corynebacteria when implicated as etiologic agents of infection.

LITERATURE CITED