Evaluation of API Coryne system for identifying coryneform bacteria

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Abstract

Aim—To identify rapidly and accurately coryneform bacteria, using a commercial strip system.

Methods—Ninety eight strains of Corynebacterium species and 62 additional strains belonging to genera Erysipelothrix, Oerskovia, Rhodococcus, Actinomyces, Arcanobacterium, Gardnerella and Listeria were studied. Bacteria were identified using conventional biochemical tests and a commercial system (API-Coryne, BioMérieux, France). Fresh rabbit serum was added to fermentation tubes for Gardnerella vaginalis isolates.

Results—One hundred and five out of the 160 (65-7%) organisms studied were correctly and completely identified by the API Coryne system. Thirty five (21-8%) more were correctly identified with additional tests. Seventeen (10-6%) organisms were not identified by the system and three (1-9%) were misidentified.

Conclusions—The system was a good alternative for identification of coryneform organisms. When occasionally performed with some additional tests, this method permits reliable and rapid identification of coryneform organisms compared with conventional methods.

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Human infections by Coryneform sp are not only increasing but microbiologists are more aware of their possible importance, mainly in high risk and immunosuppressed patients. Over the past two decades other Corynebacterium species, different from Corynebacterium diphtheriae, have been found in severe infections in people.1-3 Bacteremia, endocarditis, peritonitis, osteomyelitis and infection of the urinary and respiratory tracts are the most common infections associated with Corynebacterium sp.4-11

These bacteria, which are common in clinical samples, may be disregarded by microbiologists partly because they are considered non-pathogenic or “contaminant,” but also because there are no simple methods to identify them correctly in a routine laboratory.2 Interest is increasing in the isolation and identification of these organisms, and this led us to evaluate the API Coryne system by comparing it with conventional identification methods. This system is a micromethod for the identification of Gram positive Coryneform organisms that are aerobe or facultatively aerobe, non-sporing bacteria of the following genera: Corynebacterium, Listeria, Actinomyces, Arcanobacterium, Erysipelothrix, Oerskovia, Brevibacterium and Rhodococcus. It also permits the identification of Gardnerella vaginalis which often has a diphtheroid appearance and a variable Gram stain.

We studied 160 organisms in total from different species of the Corynebacterium genus, as well as from other morphological related genera or groups, some of them not included in the API Coryne database.

Methods

The study was carried out on Gram positive bacilli belonging to the genera Corynebacterium, Erysipelothrix, Oerskovia, Rhodococcus, Actinomyces, Arcanobacterium, Gardnerella and Listeria included in the API Coryne database (table 1). We also studied some organisms belonging to genera that occasionally present a diphtheroid appearance and are not included in the system database (table 2). A total of 160 organisms were evaluated, including 42 reference strains. Clinical isolates were obtained from blood (seven isolates), skin (17 isolates), urine (13 isolates), calcule (one isolate), drainage (one isolate), exudate (one isolate) and abscess (one isolate). The rest of the organisms came from stock collections. All the strains were kept at −70°C before use and cultivated either aerobically or, if necessary, in a CO2 atmosphere for 24 to 48 hours at 35°C on heart-infusion agar supplemented with 5% sheep blood.

The strains were identified using techniques cited by Hollis and Weaver,13 Bayston and Higgins,14 Coyle1 and others.15-17 The following tests were used: Gram staining; colony pigmentation; haemolysis; catalase production; urease utilisation; gelatin, hippurate and aesculin hydrolysis; the Voges-Proskauer reaction; nitrate reduction; acid production from glucose, maltose, mannitol, xylose, sucrose, lactose and glycogen in fermentation broth, with the addition of 10% rabbit serum for Gardnerella vaginalis. Oxidation and fermentation tests were performed for C. aquaticum strains. Casein, xanthine, and tyrosine hydrolysis were used to identify Nocardia spp. Most of the strains were identified to species level using these tests but Oerskovia spp were only identified to genus level.

The API Coryne system consists of 20 microtubes containing dehydrated substrates for the demonstration of 11 enzymatic reactions. This method is a good alternative to the conventional technique for the rapid identification of Gram positive Coryneform bacteria.
The readings, except for the aesculin, urease, and gelatin tests, were done after adding the appropriate reagents. The fermentation reactions were considered positive when they turned yellow. Identification was made using each of the tests provided by BioMérieux and, when there were difficulties, by consulting the API computer service. The interpretation was carried out adding data on macroscopic and microscopic morphology, catalase, and haemolysis, as well as the numerical profile of the API Coryne system.

All the strains with a profile of "acceptable" identification or better were considered correctly identified. Some additional tests were performed on those strains with a profile of "good identification to genus" within the group C. renale/C. cyttid and C. aquacium/Coryneform CDC group A4, according to the manufacturer's protocol. These tests included growth in 6% sodium chloride, production of acid from trehalose or fructose, the Voges-Proskauer reaction, the CAMP test, growth at a pH of 5-4 and Tween-80 hydrolysis. The strains with a profile of "low", "doubtful" or "insufficient discrimination" were considered unidentified. When the profile was good at species level but did not match the conventional identification, it was considered incorrectly identified.

### Table 2 Other species studied in the API Coryne database

<table>
<thead>
<tr>
<th>Strains</th>
<th>Total</th>
<th>Clinical Stock</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium ammonigenes</td>
<td>1</td>
<td>ATCC 6871</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium callunae</td>
<td>1</td>
<td>ATCC 15991</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium flavescens</td>
<td>1</td>
<td>ATCC 10340</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium varium</td>
<td>1</td>
<td>ATCC 10234</td>
<td></td>
</tr>
<tr>
<td>Clostridium michiganense</td>
<td>1</td>
<td>CCUG 580</td>
<td></td>
</tr>
<tr>
<td>Cortobacterium flaccumfaciens</td>
<td>1</td>
<td>CCUG 23824</td>
<td></td>
</tr>
<tr>
<td>Rhodoctococcus rhodochrous</td>
<td>1</td>
<td>ATCC 11048</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium acridum</td>
<td>2</td>
<td>ATCC 25577</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium granulosum</td>
<td>1</td>
<td>ATCC 25564</td>
<td></td>
</tr>
<tr>
<td>Rocha demarcani</td>
<td>2</td>
<td>ATCC 17931</td>
<td></td>
</tr>
<tr>
<td>Nocardia astroides</td>
<td>2</td>
<td>ATCC 19247</td>
<td></td>
</tr>
<tr>
<td>Nocardia brasiliensis</td>
<td>1</td>
<td>ATCC 19296</td>
<td></td>
</tr>
<tr>
<td>Nocardia farcinica</td>
<td>1</td>
<td>ATCC 3318</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus acidiphilus</td>
<td>2</td>
<td>ATCC 832</td>
<td></td>
</tr>
</tbody>
</table>

activities (nitrate reduction, pyrazinamidase, pyrroldinyl arylamidase, alkaline phosphatase, β glucuronidase, β galactosidase, α glucosidase, N-Acetyl-D-glucosaminidase, aesculin, urease and hydrolysis of gelatin) or the fermentation of eight sugars (glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycolen). The catalase test was performed by adding 1 drop of hydrogen peroxide (3%) to the aesculin or gelatin test. After one minute the appearance of bubbles corresponded to a positive reaction.

The inoculum was prepared in distilled water with a turbidity greater than 6 on the McFarland scale measured by comparing it with the turbidity control included in the kit. This inoculum was used for enzymatic tests. To carry out the fermentation tests, about 0.5 ml of bacterial suspension was transferred to an ampoule containing 2 ml of GP medium, with the addition of 10% rabbit serum for Gardnerella vaginalis. After homogenisation, this new suspension was distributed into the fermentation tubes and overlayed the cupules with mineral oil. The same was done for the urea hydrolysis tube. The strip was then incubated at 37°C for 24 hours. Blood agar was also incubated as a control.

The species of "related genera" included in this study were correctly identified. L. monocytogenes and L. innocua required the CAMP test and haemolysis to distinguish between them.
as the manufacturer recommends. All the *G. vaginalis* strains required 10% rabbit serum to be added to the inoculum, to be properly identified. *Oerskovia* sp required the API database.

We also included four non-pathogenic species of corynebacteria and 14 strains belonging to seven genera of aerobic, aerotolerant, or branched bacteria that can present a diphtheroid appearance and are not included in the API Coryne system database. Seventeen of these strains corresponded to profiles of "low", "doubtful" or "insufficient discrimination" or non-existent profiles and were regarded as not having been identified by the system. *Rhodococcus rhodochrous* was misidentified as *R. equi* (profile number 2151004).

**Discussion**

Over the past decades an increase in opportunistic infections in Gram positive diphtheroids has aroused interest in their identification in clinical laboratories. Conventional methods are slow and complex because of the number of tests that have to be performed which in the end can only identify between 40%-60% of the isolates.1

Previous investigations have done similar studies testing commercial systems for the study and identification of coryneforms.18-20 The API Coryne is a commercial system for the identification of aerobic or facultative, non-branched and non-spore forming Gram positive diphtheroid rods. In the clinical laboratory the isolation of organisms that have these characteristics but are not truly coryneforms is common and could be tested with the API Coryne system by mistake. This is why we included in this study 146 strains of *Corynebacterium* species and related genera as well as 14 strains of seven genera that occasionally could be mistaken for a diphtheroid.

The API Coryne system was able to identify correctly and completely 105 (65-7%) out of the total of the organisms studied. Thirty five (21-8%) more strains were correctly identified with the aid of the additional tests or the API computer database. Accordingly, the API Coryne system identified 140 out of the 160 (87-5%) strains studied.

The number of unidentified micro-organisms was 17 out of 160 (10-6%). Unidentified organisms belonged to species not contained in the database, such as non-pathogenic or plant pathogenic species of *Corynebacterium* or related genera and unrelated organisms that occasionally present a diphtheroid morphology. An *R. rhodochrous* strain was misidentified as *R. equi*. Only three (1-9%) strains out of the 160 studied were misidentified with the API Coryne system.

Our study shows that the API Coryne system produced a similar or slightly lower percentage of correctly identified organisms compared with other investigations,21-24 but it has to be remembered that many species we studied are not included in the API Coryne database. In general, most *Corynebacterium* species and related genera were correctly identified to species level with or without additional tests, as reported before.21 22 Several species required additional tests especially in those genera other than *Corynebacterium*, such as all the *Listeria* species.21

The main difference of our work is the higher number of species that are not included in the API Coryne database and were tested to challenge the system. Only one out of the 18 strains of species not included in the API Coryne database was incorrectly identified. The rest were not identified with the system, but if we had checked whether they were anaerobic or aerobic, the Gram morphology, and for acid fast bodies, they probably would have been.

It is very important to follow the manufacturer's recommendations in respect of the preparation and amount of inoculum needed. A low inoculum gives no definitive results in bacteria with a slow or difficult growth. This happened to us with *G. vaginalis* for which we had to add 10% rabbit serum to the GP medium.

We recommend that respiration, microscopic morphology (coryneform), spores; macroscopic appearances—colony size, pigmentation, and haemolysis should all be
Evaluation of API Coryne system

checked for optimal use of the API Coryne system.

The fact that it was possible to identify 140 (87.5%) or 157 (98.1%) out of the 160 studied micro-organisms with the API Coryne system, depending on the inclusion of the unidentified organisms, shows that the API Coryne system is very reliable and accurate. Most of the Gram positive bacilli isolated from clinical samples were identified in under 48 hours, compared with at least one week by standard methods, an important factor to bear in mind.

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