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Evaluation of API Coryne in Comparison with Conventional Methods for Identifying Coryneform Bacteria

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A study was performed to evaluate a new manual miniaturized system, API Coryne (API-bioMérieux, Inc., La Balme les Grottes, France), in which conventional biochemical methods were used to identify 240 isolates of coryneform and related bacteria. A total of 40% of the isolates were excluded from the study because they could not be identified by conventional methods. Identifications of the 240 isolates obtained with API Coryne showed a 97.6% concordance with conventional methods (79% after 24 h of incubation, 21% after 48 h of incubation): 158 (65.8%) isolates were identified with no further testing, and extra testing was required for 76 (31.8%) isolates. In three (1.2%) cases, the organisms did not correspond to any key in the code book and could not be identified by the computer service of the manufacturer. Only three (1.2%) strains were misidentified. The system was shown to be reliable and rapid when compared with standard identification methods.

The incidence of opportunistic infections arising from corynebacteria is increasing, but techniques for treating high-risk patients, immunosuppressed and others, are being improved. In recent years, a number of studies have shown that coryneforms other than *Corynebacterium diphtheriae* were involved in severe human infections such as bacteremia, endocarditis, osteomyelitis, and infections of the respiratory tract (5, 17, 18, 33). Coryneform infections generally occur in immunosuppressed patients after catheters or heart valves have been fitted.

These bacteria, which are isolated more and more frequently, are nonetheless little known by bacteriologists, largely because there are no simple methods to correctly identify them in a routine laboratory. The aim of this study was to describe and evaluate the API Coryne system (API-bioMérieux, Inc., La Balme les Grottes, France), which was designed for the identification of gram-positive bacteria, mostly of the *Corynebacterium* type.

MATERIALS AND METHODS

Bacteria. The study was carried out on gram-positive bacilli belonging to the genera *Corynebacterium*, *Erysipelothrix*, *Oerskovia*, *Rhodococcus*, *Actinomyces*, *Arcanobacterium*, *Brevibacterium*, and *Listeria* (Table 1). A total of 240 organisms were evaluated, including 22 reference strains and 218 isolates from clinical specimens. Clinical isolates were obtained from the following sources: blood (18 isolates), cerebrospinal fluid (1 isolate), wounds (114 isolates), the otorhinolaryngological area (37 isolates), the genital tract (9 isolates), urine (24 isolates), and unknown (15 isolates). The strains were cultivated either aerobically or, if necessary, in a CO_2 atmosphere for 24 to 48 h at 35°C on Trypticase-soy agar supplemented with horse blood (bioMérieux).

Conventional identification. The strains were identified by using many of the tests cited by Weaver and Hollis (30), Jones and Collins (14), and Estrangin et al. (9). The following tests were used: Gram staining; colony pigmentation; horse blood hemolysis; catalase; motility; urea; gelatin and esculin hydrolysis; acetoin production; nitrate reduction; β-galactosidase; alkaline phosphatase; glucose oxidization and fermentation; and fermentation of sucrose, fructose, mannose, maltose, trehalose, mannitol, lactose, raffinose, and xylose. By use of these methods for phenotypic identification, only 60% of random clinical isolates were characterized and retained for comparative study. Moreover, *Brevibacterium*, *Oerskovia*, and *Rhodococcus* species were characterized by using other criteria, such as examination of percent G+C (21), mycolic acid (8), menaquinone (12, 20), and cell wall sugar (7) contents. *Brevibacterium* and *Oerskovia* species were identified only to the genus level.

API Coryne strip system. With the API Coryne strip system, 21 biochemical tests can be performed in 24 or 48 h. The strip consists of 20 tubes containing dehydrated substrates. These substrates allow for 11 enzyme tests (pyrazinamidase, pyrrolidonyl arylamidase, β-galactosidase, alkaline phosphatase, α-glucosidase, *N*-acetylglucosaminidase, β-glucuronidase, and nitrate reduction and gelatin, urea, and esculin hydrolysis) and 8 carbohydrate fermentation tests (glucose, ribose, D-xylose, mannitol, maltose, lactose, sucrose, and glycogen as well as a negative test fermentation). Catalase activity was determined by adding a drop of hydrogen peroxide (3%) to one of the two tubes corresponding to the esculin or gelatin hydrolysis tests.

The inoculum was prepared from a heavy suspension in distilled water with an opacity of more than 6 on the McFarland scale. The inoculum was used for enzymatic tests. In order to carry out the fermentation tests, 0.5 ml of the suspension described above was added to 2 ml of enriched medium containing phenol red pH indicator (GP medium). After homogenization, the medium was placed into the different tubes. In order to ensure anaerobiosis, mineral oil was added to the tubes used for urea hydrolysis, sugar fermentations, and the negative control.

The strip was then incubated at 35°C for 24 or 48 h. A control Trypticase-soy agar tube supplemented with horse blood was used in parallel with strip inoculation. When growth on blood gelose was adequate after 24 h, the test strip

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TABLE 1. Distribution of API Coryne identifications

Organism identified by API Coryne	No. of strains					Misidentification
	Tested	Correctly identified	Correctly identified with extra tests	Not identified	Incorrectly identified one choice	
"*Corynebacterium aquaticum*"	5	3	2			
Corynebacterium group A4	5	5				
Corynebacterium group A5	3	1	2[a]			
"*Corynebacterium bovis*"	2	1				
Corynebacterium diphtheriae subsp. *mitis*	1				1	*Corynebacterium diphtheriae* subsp. *gravis*
Corynebacterium diphtheriae	4	4				
Corynebacterium diphtheriae subsp. *belfanti*	1	1				
Corynebacterium diphtheriae subsp. *gravis*	1	1				
Corynebacterium jeikeium	34	22	12			
Corynebacterium minutissimum	32	18	14			
Corynebacterium pseudotuberculosis	1	1				
Corynebacterium pseudodiphtheriae	18	15	3			
Corynebacterium renale	2	2				
Corynebacterium striatum	58	31	27			
Corynebacterium ulcerans	3	3				
Corynebacterium xerosis	9	8			1	*Corynebacterium striatum*
Corynebacterium group D2	14	14				
Corynebacterium group F2	8	8				
Corynebacterium group G1	6	4	2			
Corynebacterium group G2	3	3				
Corynebacterium group I1	1	1				
Corynebacterium group I2	7	7				
Rhodococcus equi	4		3	1		
Listeria monocytogenes	4		4			
Listeria innocua	1		1			
Oerskovia spp.	3		2[a]	1		
Actinomyces pyogenes	2	2				
Erysipelothrix rhusiopathiae	1			1		
Arcanobacterium haemolyticum	3	3				
Brevibacterium spp.	4		3[a]		1	*Corynebacterium* group B
Total	240	158	76	3	3	

[a] No extra tests suggested by the manufacturer.

results were read. When growth was not sufficient, the strips were incubated for a further 24 h before the results were read.

Apart from the esculin, urea, and gelatin hydrolysis tests, for which an immediate reading was possible, results were obtained for the enzymatic tests after appropriate reagents were added. The sugar fermentation reactions were considered positive when the pH indicator turned yellow.

Analysis of the data was carried out with the help of APILAB software by using the API Coryne (version 1.0) data base. A profile index was also available.

RESULTS

Of the 240 strains tested by the API Coryne system, results for 234 (97.6%) of them were in concordance with results of conventional methods; 158 (65.8%) were identified with no further tests, and additional tests were needed for identification of 76 (31.8%) isolates. In three (1.2%) cases, the organisms did not correspond to any key, and three (1.2%) strains were misidentified. Most species, in particular those often found in the clinical laboratories (18), were correctly identified by the system. Of these, *Corynebacterium pseudodiphtheriticum* (15 of 18 isolates), *Corynebacterium xerosis* (8 of 9 isolates), and *Corynebacterium* group D2 (14 of 14 isolates) were almost invariably correctly identified by the system. Other bacteria also frequently encountered, such as *Corynebacterium jeikeium* (22 of 34 strains correctly identified), *Corynebacterium minutissimum* (18 of 32 strains correctly identified) and *Corynebacterium striatum* (31 of 58 strains correctly identified) often required additional tests. The extra tests suggested by the manufacturer for strains not identified directly by the API system are listed in Table 2.

A *Brevibacterium* strain was interpreted as *Corynebacterium* group B because of a positive reaction to the α-glucosidase and alkaline phosphatase tests. Of the nine *C. xerosis* strains tested, one was identified as *Corynebacterium striatum* because of a weak nitrate reaction, which was interpreted as negative. The three unidentified strains belonged to *Rhodococcus equi* and *Erysipelothrix rhusiopathiae* and the genus *Oerskovia*.

Identification was usually carried out after 24 h of incubation, except for bacteria such as *C. jeikeium*, *Corynebacterium bovis*, and *Corynebacterium* group G (21% of strains), which required a further 24 h of incubation.

DISCUSSION

The fact that only 60% of the gram-positive bacilli routinely isolated in the clinical laboratory can be identified by conventional phenotypic methods reveals the problems that are encountered when studying this group of bacteria.

In the same manner, the API Coryne system also identified most of the species of these well-characterized coryne-

TABLE 2. Extra tests suggested by the manufacturer for strains not identified directly by the API system

Organism (no. of strains)	Extra test (no. of strains tested)
Corynebacterium aquaticum (2)	Pigment (2) Motility (2)
Corynebacterium bovis (1)	Pigment (1) CAMP test (*Staphylococcus aureus*) (1) NaCl 6% (1) Tween 80 (1)
Corynebacterium jeikeium (12)	NaCl 6% (12) Fructose (acid) (12) Tween 80 (2)
Corynebacterium minutissimum (14)	NaCl 6% (14) Fructose (acid) (14)
Corynebacterium pseudodiphtheriticum (3)	CAMP test (*Staphylococcus aureus*) (3) Tween 80 (3) Pigment (3)
Corynebacterium striatum (27)	42°C (27)
Corynebacterium group G1 (2)	42°C (2)
Rhodococcus equi (3)	Pigment (3) 42°C (2) CAMP test (*Staphylococcus aureus*) (3)
Listeria monocytogenes (4)	Hemolysis (4) CAMP test (*Staphylococcus aureus*) (4)
Listeria innocua (1)	Hemolysis (1) CAMP test (*Staphylococcus aureus*) (1)

form bacteria, with or without extra tests (Table 2). The API strip is a reliable system for identification of *C. diphtheriae* species but not for its subspecies: *C. diphtheriae* subsp. *gravis*, *C. diphtheriae* subsp. *mitis*, and *C. diphtheriae* subsp. *belfanti*. We therefore suggest that these subspecies be deleted from the data base, keeping only *C. diphtheriae*. Besides, some species, such as *C. diphtheriae* subsp. *belfanti*, *C. diphtheriae* subsp. *gravis*, *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Corynebacterium ulcerans*, and *Corynebacterium* groups G2 and I1, although correctly identified, were represented by an insufficient number of strains to determine the reliability of the system.

Species of gram-positive bacteria other than corynebacteria, *Actinomyces pyogenes* and *Arcanobacterium haemolyticum*, were correctly identified without further tests. The *Listeria*, *Brevibacterium*, and *Oerskovia* species were identified only after the use of extra tests. The API strip allows only presumptive identification of these bacteria to the genus level. Moreover, among the extra phenotypic tests proposed by the manufacturer, none allows for the definitive characterization of *Oerskovia* and *Brevibacterium* species, and it appeared to be difficult to avoid the performance of chemotaxonomic tests such as the study of cell wall constituents (mycolic acids, fatty acids, menaquinone, and amino acids) (14, 16, 22). It also proved impossible to identify the *Erysipelothrix rhusiopathiae* strain.

Some corynebacteria have clearly established pathogenicities. This is the case for *C. diphtheriae*, as well as *Corynebacterium* group D2 which is found more and more frequently in the clinical field and is often responsible for urine infections and lithiasis (27). *C. jeikeium* is another pathogenic corynebacterium which has been involved in prosthetic endocarditis, meningitis, and several other diseases (6, 24). These last two species are characterized by their remarkable resistance to most antibiotics (10, 24, 25). The pathogenicities of the other corynebacteria remain uncertain (3). It appears to be linked to the difficulty in correctly identifying these microorganisms. The reference methods for identification of coryneform and related bacteria use techniques available only to specialized laboratories, such as the study of wall and genome constituents (14), which are not easy to run in routine laboratories. The method derived from chemotaxonomic studies, such as that developed by Athalye et al. (1) in which gas-liquid chromatography is used, is also difficult to use, since it requires a 2- to 3-day culture in an enriched medium. This is equally true for techniques involving protein electrophoresis (13).

In the same way, conventional phenotypic methods, such as those described by Weaver and Hollis (30), require too many tests for easy use in a routine clinical microbiology laboratory. A number of simplified methods have been suggested, such as those using an API Staph strip (2), an API 20 Strep strip (2, 15, 19, 29), the Minitek system (26), the RIM (Rapid Identification Method) (11), and the rapid identification strip developed by Thompson et al. (28). However, these systems have been tested with a relatively limited number of bacterial species, mostly those of recognized clinical interest, such as *C. diphtheriae*, *Corynebacterium* group D2, and *C. jeikeium*. Likewise, selective media (2, 4, 23, 31) and some specific tests proposed previously (32) are restricted to limited groups of microorganisms.

The API Coryne strip system described in this report has been specially developed for identifying gram-positive rods, mainly coryneform bacteria, and seems well-adapted to routine work. It is an improved system for the characterization of most of the gram-positive bacteria frequently isolated in human clinical samples, because it is easy to use, its performance is equal to those of standard methods, and identification is rapidly obtained. For some genera and species, it is not easy to do without chemotaxonomic or genomic characterization methods. On the other hand, a study is under way to evaluate the test strip with regard to strains that cannot be identified by phenotypic methods. The simplicity, accuracy, and speed of the system will provide many laboratories with an important confirmatory test method.

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