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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, Archanobacterium, Gardnerella* and *Listeria* were studied. Bacteria were identified using conventional biochemical tests and a commercial system (API-Coryne, BioMèrieux, 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] Bacteraemia, 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-spore forming organisms 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 CO_2 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] Coyle[1] 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

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Table 1 Corynebacterium species and related genera studied included in the API Coryne database

Strains	*Total*	*Clinical*	*Stock*	*Reference*
Corynebacterium urealyticum	27	22	2	ATCC 43042, ATCC 43043, ATCC 43044
Corynebacterium jeikeium	22	15	5	ATCC 43734, CCUG 24871
Corynebacterium striatum	5		4	ATCC 6940
Corynebacterium xerosis	5		4	ATCC 373
Corynebacterium diphtheriae	2		2	
Corynebacterium pseudotuberculosis	4		3	ATCC 19410
Corynebacterium bovis	2			ATCC 7715, CCUG 2705
Corynebacterium pseudodiphtheriticum	5		4	ATCC 10700
Corynebacterium kutscheri	2		1	ATCC 15677
Corynebacterium renale	2		1	ATCC 19412
Corynebacterium cystitidis	1			ATCC 29593
Corynebacterium pilosum	1			ATCC 29592
Corynebacterium minutissimum	2			ATCC 23348, CCUG 541
Corynebacterium ulcerans	6		4	CCUG 16556, NCTC 7907
Corynebacterium aquaticum	3		2	ATCC 14665
Coryneform CDC group F_1	3	1	2	
Coryneform CDC group A_4	2		2	
Coryneform CDC group G_2	1		1	
Oerskovia sp	4		3	ATCC 25835
Erysipelothrix rhusiopathiae	4		4	
Rhodococcus equi	8	1	7	
Listeria monocytogenes	11		9	ATCC 19111, NCTC 11994
Listeria innocua	2		2	
Listeria murrayi	1			ATCC 25401
Listeria grayi	1			ATCC 25400
Listeria ivannovii	1		1	
Listeria seeligeri	2		2	
Arcanobacterium haemolyticum	5		4	ATCC 9345
Actinomyces pyogenes	4		3	ATCC 19411
Gardnerella vaginalis	4	1	2	ATCC 14018

Table 2 Other species studied not included in the API Coryne database

Strains	*Total*	*Clinical*	*Stock*	*Reference*
Corynebacterium ammoniogenes	1			ATCC 6871
Corynebacterium callunae	1			ATCC 15991
Corynebacterium flavescens	1			ATCC 10340
Corynebacterium vitarumen	1			ATCC 10234
Clavibacter michiganense	1			CCUG 580
Curtobacterium flaccumfaciens	1			CCUG 23824
Rhodococcus rhodochrous	1			ATCC 11048
Propionibacterium avidum	2	1		ATCC 25577
Propionibacterium granulosum	1			ATCC 25564
Rothia dentocariosa	2		1	ATCC 17931
Nocardia asteroides	2		1	ATCC 19247
Nocardia brasiliensis	1			ATCC 19296
Nocardia farcinica	1			ATCC 3318
Lactobacillus acidophilus	2		1	ATCC 832

activities (nitrate reduction, pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β glucuronidase, β galactosidase, α glucosidase, N-Acetyl-βglucosaminidase, aesculin, urease and hydrolysis of gelatin) or the fermentation of eight sugars (glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycogen). 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 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 the table provided by BioMèrieux and, when there were difficulties, by contacting 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 cystitidis* and *C aquaticum*/ Coryneform CDC group A, 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.

Results

Of the 160 organisms studied using the API system, 105 (65·7%) of them were correctly and completely identified in 24 hours to species level, 35 more (21·8%) were incompletely identified but finally correctly identified with additional tests, 17 (10·6%) were not identified as the profile number did not correspond to any organism, and in three (1·9%) cases the strains were misidentified. Most strains (87·5%) were correctly identified in 24 hours or after additional tests (table 3).

Six strains of *C jeikeium* had a good profile at genus level (profile number 2100324) and may represent a less common biotype. Additional tests were required for correct identification (growth in 6% sodium chloride and the production of acid from fructose). One strain of *C striatum* required the Voges-Proskauer test as an additional test to differentiate it from Coryneform CDC group G_2. None of the *C aquaticum* strains was correctly and completely identified by the API system. One of them required growth at 42°C and oxidation or fermentation tests as additional tests. The other two were misidentified as *R equi* and *Listeria* spp. The strains belonging to the group *C renale/C cystitidis* are indistinguishable using the API system, as they have the same profile. Additional tests such as growth in pH 4·5 and Tween-80 hydrolysis were required to differentiate both of them. All the Coryneform CDC groups were correctly identified in 24 hours.

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,

Table 3 Distribution of API Coryne identification

Organisms studied	Tested	Correctly and completely identified	Incompletely but correctly identified	Not identified	Incorrectly identified
Corynebacterium urealyticum	27	27			
Corynebacterium jeikeium	22	16	6		
Corynebacterium striatum	5	4	1		
Corynebacterium xerosis	5	5			
Corynebacterium diphtheriae	2	2			
Corynebacterium pseudotuberculosis	4	4			
Corynebacterium bovis	2	2			
Corynebacterium pseudodiphtheriticum	5	5			
Corynebacterium kutscheri	2	2			
Corynebacterium renale	2	2			
Corynebacterium cystitidis	1	1			
Corynebacterium pilosum	1	1			
Corynebacterium minutissimum	2	2			
Corynebacterium ulcerans	6	6			
Corynebacterium aquaticum	3		1		2
Coryneform CDC group F_1	3	1	2		
Coryneform CDC group A_4	2	2			
Coryneform CDC group G_2	1	1			
Oerskovia sp	4	2	2		
Erysipelothrix rhusiopathiae	4	4			
Rhodococcus equi	8	8			
Listeria monocytogenes	11		11		
Listeria innocua	2		2		
Listeria murrayi	1		1		
Listeria grayi	1		1		
Listeria ivannovii	1		1		
Listeria seeligeri	2		2		
Arcanobacterium haemolyticum	5	4	1		
Actinomyces pyogenes	4	4			
Gardnerella vaginalis	4		4		
Corynebacterium ammoniogenes	1			1	
Corynebacterium callunae	1			1	
Corynebacterium flavescens	1			1	
Corynebacterium vitarumen	1			1	
Clavibacter michiganense	1			1	
Curtobacterium flaccumfaciens	1			1	
Rhodococcus rhodochrous	1				1
Propionibacterium avidum	2			2	
Propionibacterium granulosum	1			1	
Rothia dentocariosa	2			2	
Nocardia asteroides	2			2	
Nocardia brasiliensis	1			1	
Nocardia farcinica	1			1	
Lactobacillus acidophilus	2			2	
	160	105(65·7%)	35(21·8%)	17(10·6%)	3(1·9%)
		140(87·5%)			
			157(98·1%)		

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 by 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 aerobe 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

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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