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Identification of Clinically Relevant *Corynebacterium* spp., *Arcanobacterium haemolyticum*, and *Rhodococcus equi* by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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The identification of 83 *Corynebacterium*, 13 *Arcanobacterium haemolyticum*, and 10 *Rhodococcus equi* strains by conventional methods (API Coryne complemented with 16S rRNA gene sequence analysis) was compared with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry identification. The correlation between API and MALDI-TOF results was 89%. MALDI-TOF is a rapid and accurate system for identification of the above-mentioned microorganisms.

Corynebacteria are widespread throughout nature. Pathogenic *Corynebacterium* species include *Corynebacterium diphtheriae* and nondiphtheroid *Corynebacterium*. The nondiphtheroid *Corynebacterium* species are found in the mucosa and normal skin flora of humans and animals. Some species, such as *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, *Corynebacterium striatum*, *Corynebacterium urealyticum*, and *Corynebacterium xerosis*, are relevant human pathogens, mainly infecting immunocompromised patients (4). The API Coryne V2.0 system (bioMérieux, Marcy l'Etoile, France), complemented with conventional phenotypic testing, is most commonly used in routine laboratories for identification of these microorganisms. However, this method is time-consuming and does not always give reliable identification at the species level (2). Identification by means of 16S rRNA sequencing is more specific but is slow and expensive.

Arcanobacterium haemolyticum is an obligate parasite of the pharynx of humans; sporadically, it causes pharyngeal or skin lesions (11). *Rhodococcus equi* is the most important pathogenic species of the genus *Rhodococcus*, causing several infections such as necrotizing pneumonia and enteritis, mainly in immunocompromised patients such HIV-positive patients (14). *A. haemolyticum* and *R. equi* are also routinely identified using the API Coryne V2.0 system. In the case of *R. equi*, reliable identification frequently requires confirmation by molecular methods, including PCR and DNA sequencing (7).

In recent years, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been increasingly applied in clinical microbial diagnostics for species identification of bacterial and fungal pathogens (13). MALDI-TOF MS identified successfully at the species level a group of 115 *Corynebacterium* clinical isolates, including 78 *C. diphtheriae* and 37 nondiphtheroid corynebacterial isolates (6). In a recent study (3), MALDI-TOF discriminated *Corynebacterium aurimucosum* from *Corynebacterium minutissimum*, two closely related *Corynebacterium* species previously considered difficult to differentiate (5).

The aim of this study was to determine whether MALDI-TOF MS can be used as a routine method for fast and reliable identification of *Corynebacterium* clinical isolates at the species level in our laboratories. Eighty-three clinical strains isolated at the Clinical Microbiology Laboratory, Hospital Universitario Marqués de Valdecilla, Santander, Spain, were initially identified by API Coryne V2.0 and other conventional phenotypic methods (4) as *C. jeikeium* (18 strains), *Corynebacterium pseudodiphthericum* (16 strains), *C. striatum* (15 strains), *C. amycolatum* (12 strains), *C. urealyticum* (9 strains), *Corynebacterium glucuronolyticum* (3 strains), *Rhodococcus equi* (11 strains), and *Arcanobacterium haemolyticum* (13 strains). The strains *C. striatum* ATCC 6940 and *C. urealyticum* ATCC 43042 were also included in the study. Toxin-producing species such as *Corynebacterium diphtheriae* were not examined in this study because of a lack of samples in this particular collection. The strains were analyzed by MALDI-TOF MS through the following procedure: a small amount of a colony grown on blood agar was transferred to a metallic MALDI-TOF MSP 96 plate (Bruker Daltonik GmbH, Bremen, Germany) and thereafter covered with 1 μl of matrix (saturated alpha-cian-4-hydroxycinamic acid–50% acetonitrile–2.5% trifluoroacetic acid). The plate was then left to dry at room temperature for 5 min. When that first attempt at identification by MS did not show results with a high level of confidence, samples were identified using formic acid-acetonitrile extraction. The reference strain *Escherichia coli* K-12 (genotype GM48) was used as a standard for calibration and as a reference for quality control. Measurements were performed using a Microflex II mass spectrometer (Bruker Daltonik) equipped with a 60-Hz laser. The proteic spectra obtained with the spectrometer were processed with Bruker MALDI Biotyper v2.0 software (Bruker Daltonik GmbH) and compared with the reference spectra present in the database, showing the 10 most

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TABLE 1 Comparative results of the identification methods used in this study

Microorganism	Total no. of strains	No. of strains identified by indicated method		
		API Coryne V2.0	MALDI-TOF	16S rRNA sequencing
Corynebacterium amycolatum	12	12	12	
Corynebacterium glucuronolyticum	3	3	3	
Corynebacterium jeikeium	19	19	18 *C. jeikeium*	
			1 *C. amycolatum*	1 *C. amycolatum*
Corynebacterium minutissimum	7	7	3 *C. minutissimum*[a]	
			3 *C. aurimucosum*	3 *C. aurimucosum*
			1 *C. amycolatum*	1 *C. amycolatum*
Corynebacterium pseudodiphtheriticum	16	16	11 *C. pseudodiphtheriticum*[b]	
			5 *C. propinquum*[a]	5 *C. propinquum*
Corynebacterium striatum	16	16	16	
Corynebacterium urealyticum	10	10	10	
Rhodococcus equi	11	11	10 *R. equi*	
			1 *Dietzia maris*	1 *D. maris*
Arcanobacterium haemolyticum	13	13	13	

[a] Two of these three strains were identified as *C. aurimucosum* by sequencing the *rpoB* gene.
[b] All *C. pseudodiphteriticum* and *C. propinquum* isolates identified by MALDI-TOF MS were confirmed by sequencing the *rpoB* gene.

similar patterns for each isolate. The similarity of patterns is demonstrated as a score (≥2, identification at species level; 1.7 to 1.999, identification at genus level; <1.7, no reliable identification). Discrepancies between API Coryne V2.0 and MALDI-TOF MS identification were resolved by partial sequencing of the 16S rRNA gene and sequencing of the *rpoB* gene (1).

All the isolates identified as *C. amycolatum*, *C. glucuronolyticum*, *C. striatum*, *C. urealyticum*, and *A. haemolyticum* by API Coryne V2.0 were also identified by MALDI-TOF MS as such, scoring ≥2 (Table 1). Of 19 strains identified as *C. jeikeium* by API Coryne V2.0 testing, 1 was identified as *C. amycolatum* by MALDI-TOF MS. 16S rRNA sequencing assigned this isolate to *C. amycolatum*. *C. jeikeium* is recognized as one of the *Corynebacterium* species most frequently associated with human infectious diseases (8), causing sepsis, endocarditis, pneumonia, and other infections, while *C. amycolatum* is considered an emergent pathogen (9). Of 7 strains identified as *C. minutissimum* by API Coryne V2.0, 3 were also identified by MALDI-TOF MS, 3 were assigned to *C. aurimucosum*, and 1 was assigned to *C. amycolatum* (Table 1). 16S rRNA sequencing confirmed that the isolates did indeed belong to the species identified by MALDI-TOF. However, when the *rpoB* gene was sequenced, the results obtained by MALDI-TOF were confirmed, with the exception of two *C. minutissimum* strains, which were identified as *C. aurimucosum*; therefore, further investigation is needed to evaluate the use of MALDI-TOF MS to identify this species. It has been previously reported that, because their biochemical profiles are very similar, API Coryne V2.0 did not discriminate among these three species, needing complementary tests for a reliable identification (12). Discrepancies between API Coryne V2.0 and MALDI-TOF MS results were also found with 5 of the 16 strains identified as *C. pseudodiphtheriticum* by API Coryne V2.0, all 5 of which were assigned to *C. propinquum* by MALDI-TOF MS. 16S rRNA sequencing confirmed that the 5 isolates were *C. propinquum*. Indeed, there was also total correlation between the identification by MALDI-TOF MS and sequencing of the *rpoB* gene. *C. pseudodiphthericum* and *C. propinquum* are part of the oropharyngeal microbiota. They have been reported to be among the species causing pneumonia and endocarditis. The two species are phenotypically analogous, but most *C. pseudodiphthericum* strains produce urease, whereas *C. propinquum* strains are urease negative.

Of 11 isolates presumptively identified as *R. equi* by API Coryne V2.0, MALDI-TOF MS identified all except 1, which was identied as *Dietzia maris*. 16S rRNA gene sequencing identified this isolate as *D. maris*. We have recently reported that 8 of 15 clinical isolates presumptively identified by API Coryne as *R. equi* at Hospital Marqués de Valdecilla, Santander, Spain, belonged in fact to the genus *Dietzia* (10).

Since most of the nondiphtheroid strains of *Corynebacterium*, *A. haemolyticum*, and *R. equi* are actually well-recognized human pathogens, their reliable identification at the species level is necessary. API Coryne V2.0 is, at this time, the method most commonly used for identification of these bacteria in routine microbiological laboratories, but its trustworthiness at the species level is limited. Moreover, it requires at least 16 h for processing after isolation of suspicious colonies from screening plates. In contrast, MALDI-TOF MS analysis is much faster, rendering species identification of one isolate in less than 10 min. This study demonstrates that MALDI-TOF MS is a rapid and consistent system for routine identification at the species level of clinical isolates of the above-mentioned microorganisms.

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