Letter to the editor

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Evaluation of the VITEK-MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for the identification of clinical *Corynebacterium* species

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Sir,

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a fast, highly accurate and cost-effective method in clinical microbial diagnostics for identification of bacterial and fungal pathogens[1]. The two MS-based more frequently used bacterial identification systems are the VITEK-MS (bioMérieux, Marcy-l'Étoile, France) and the MALDI Biotyper (Bruker Daltonics GmbH, Bremen, Germany). Corynebacteria are widely distributed in nature. Pathogenic *Corynebacterium* species include *Corynebacterium diphtheriae* and nondiphtheroid *Corynebacterium*. Non-diphtherial corynebacteria are part of the normal skin and mucous membranes flora of humans. They are recognized as opportunistic pathogens[2], particularly in immunocompromised patients. These microorganisms are routinely identified with the API Coryne 2.0 system (bioMérieux) and complementary phenotypic tests. However this method is time-consuming and does not always give reliable identification at the species level. Identification by means of 16S rRNA and *rpoB* gene sequencing is more specific but is slow and expensive[3]. The MALDI Biotyper has been successfully applied for accurate identification at the species level of *Corynebacterium* spp. clinical isolates[4]. However it could not reliably differentiate neither *C. aurimucosum* from *C. minutissimum*[4,5] nor *C. minutissimum* from *C. singulare*[5]. *C. aurimucosum* causes urinary tract infections in males and females whereas *C. minutissimum* is considered the causative agent of the chronic skin condition erythrasma. We aimed to evaluate the performance of the VITEK-MS system for identification at the species level of a collection of *Corynebacterium* spp. clinical isolates, comparing the results with those previously obtained using the MALDI Biotyper[5]. Ninety-five aerobic coryneform bacteria obtained from clinical specimens collected during the period 2007-2012 at the clinical microbiology laboratory of the University Hospital F. Hached, Sousse, Tunisia, were included in this study. The 95 coryneform bacteria were isolated from surgical wound exudates, non-surgical wound exudates, blood, urine, stool, and respiratory tract specimens. The collection included the species *C. striatum, C. amycolatum, C. aurimucosum, C. singulare, C. simulans, C. imitans, C. tuberculostearicum, C. mucifaciens, C. diphtheriae, C. coylae, C. macginleyi* and *C. jeikeium*, as they were previously identified by API Coryne and sequencing of the *rpoB* gene[5]. They were grown on Columbia horse blood agar plates at 37ºC. For VITEK-MS analysis, samples were prepared according to the manufacturer's instructions. A portion of a fresh colony was applied as a thin film on an individual spot of the 48-wells disposable target slide (bioMérieux), re-suspended in 0.5 µl of 28.9% formic acid solution (VITEK MS-FA) and allowed to visible dried at room temperature. Subsequently, 1µl of VITEK-MS α-Cyano-4-hydroxycinnamic acid (CHCA) matrix solution was applied on each sample spot. Each microorganism analyzed was deposited twice on the same target slide. After drying, the target plate was loaded onto the VITEK-MS mass spectrometer. Measurements were performed according to the manufacturer's suggested settings using automated collecting spectra. Captured spectra were analyzed with the Shimadzu Launchpad identification software that included database SARAMIS MS-ID version 2.0 (Anagnos Tee GMBH, bioMérieux). *Escherichia coli* ATCC 8739 was included as standard to calibrate the instrument and validate the run. The system reported the best identification matches along with confidence values from 0% to 99.9%. Peak matches that yield identification results with confidence values exceeding 80% were considered significant and displayed. For identification at the species level, a score value of 99.9% was required. In cases of low discrimination, two results were provided for the same sample. These cases were resolved by sequencing of the *rpoB* gene. VITEK-MS provided accurate identification at the species level for 80 (84.21%) of the 95 isolates. VITEK-MS clearly identified two strains as *C. aurimucosum* (scores 99.9%) and one

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Table 1 **Results of identification by Vitek-MS compared with that provided by MALDI Biotyper. Discrepancies were resolved by *rpoB* gene sequencing.**

	Identities given by	
rpoB sequence	VITEK-MS	MALDI Biotyper
70 *C. striatum*	70 *C. striatum*	70 *C. striatum*
2 *C. aurimucosum*	2 *C. aurimucosum*	2 *C. aurimucosum/C. minutissimum*
1 *C. singulare*	1 *C. singulare*	1 *C. singulare/C. minutissimum*
1 *C. simulans*	1 *C. simulans*	1 *C. simulans*
14 *C. amycolatum*	15 *C. amycolatum/C. xerosis*	14 *C. amycolatum*
1 *C. imitans*		1 *C. imitans*
1 *C. tuberculostearicum*	1 *C. tuberculostearicum*	1 *C. tuberculostearicum*
1 *C. mucifaciens*	1 *C. mucifaciens*	1 *C. mucifaciens*
1 *C. diphtheriae*	1 *C. diphtheriae*	1 *C. diphtheriae*
1 *C. coylae*	1 *C. coylae*	1 *C. coylae*
1 *C. macginleyi*	1 *C. macginleyi*	1 *C. macginleyi*
1 *C. jeikeium*	1 *C. jeikeium*	1 *C. jeikeium*

strain as *C. singulare* (score 99.9%) (table 1). *C. singulare* is a resident of the human skin that has also been recovered from a blood specimen and a semen specimen from two patients[6]. *C. singulare* is so closely-related to *C. minutissimum* that their 16S rDNA sequences are >98% identical. For fifteen (15.79%) out of our 95 *Corynebacterium*, VITEK-MS could not discriminate between *C. amycolatum* and *C. xerosis* (scores 50 and 50 %, respectively). *C. amycolatum* causes septicemia, frequently associated with venous catheter devices, and has also been recovered from urinary tract infections and mixed flora abscesses[2]. The species *C. xerosis* is an infrequently reported human pathogen, with isolates being identified in cases of ear infections, brain abscesses and osteomyelitis[2]. Differentiation between *C. amycolatum* and *C. xerosis* in the routine clinical laboratory is difficult since the colonial appearances are very similar and biochemical tests give variable results for different strains of the two species[7]. Fourteen of the 15 strains were assigned to *C. amycolatum* and one to *C. imitans* when they were identified by *rpoB* gene sequence analysis. *C. imitans* is rarely recovered from human clinical material and no more than 10 isolates has been reported in the literature[8]. The MALDI Biotyper clearly distinguished among *C. amycolatum*, *C. xerosis* and *C. imitans*[5].

We conclude that the VITEK-MS system provided lower accuracy (84.21%) than the MALDI Biotyper system (96.84%) for identification at the species level of our collection of 95 *C. striatum*. However, VITEK-MS could correctly identify *C. aurimucosum, C. minutissimum* and *C. singulare*.

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CONFLICT OF INTEREST

The authors declare that they have not conflict of interest concerning this article.

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