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Short Communication

Rapid Identification of *Cardiobacterium hominis* by MALDI-TOF Mass Spectrometry during Infective Endocarditis

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SUMMARY: We report a new case of *Cardiobacterium hominis* endocarditis identified during an acute coronary syndrome. The positivity of the blood cultures was confirmed rapidly (50 h) as a result of improvements to the automated detection system, whereby it is no longer necessary to incubate the vials for long periods of time when *Aggregatibacter-Cardiobacterium-Eikenella-Kingella* infections is suspected. The phenotype-based VITEK 2 NH identification system is not able to distinguish between the two species of *Cardiobacterium*, as it does not contain *C. valvarum* in its library. The method for 16S rRNA gene sequence analysis is able to separate the two species but is not available in all laboratories. We used MALDI-TOF mass spectrometry, as an alternative, to rapidly distinguish between *C. hominis* and *C. valvarum*, because both species are contained in the system library.

A 60-year-old man, without coronary history, was hospitalized in the cardiologic ward for an atypical chest constrictive pain. Initial examination showed that his pulse was regular at 80/min and that his blood pressure was 140/70 mm Hg. He had a diastolic murmur at the left sternal border with hyperpulsability of the peripheral arteries. His body temperature was 37.3°C. There were no symptoms of heart failure or peripheral cutaneous endocarditis symptoms, nor was the spleen palpable. Transesophageal echocardiography confirmed significant aortic insufficiency and showed a large valvular vegetation on a right coronary aortic cusp with prolapse into the left ventricular outflow tract. There was no periannular abscess. The C-reactive protein concentration was 24 mg/L (normal range <6 mg/L). The leukocyte count was 9.1×10^9/L, 75% of which was polymorphonuclear, the hemoglobin level was 11.8 g/dL, and the platelet count was 170×10^9/L. Four blood culture sets were collected using BacT/Alert (bioMérieux, Marcy l'Etoile, France) before starting antibiotic therapy with amoxicillin (12 g/d), gentamycin (240 mg/d), and levofloxacin (750 mg/d). The four aerobic blood cultures were found to be positive after 50 h of incubation. The diagnosis of endocariditis was confirmed on the basis of a positive direct examination of blood culture using a pleiomorphic Gram-negative rod. Before the antibiogram was available, the therapy was modified: amoxicillin was replaced with cefotaxime (7 g/d). The infectious process was favorable, and the aortic insufficiency was treated using a mechanical prosthesis. The culture obtained from the native valve was sterile. The history of this patient, after re-examination, revealed a dermatological lesion on the right arm treated with corticoids (prednisolone) per os 15 days before the beginning of the illness.

Direct examination showed that the morphology of this Gram-negative rod was pleiomorphic, and pairs, short chains, and filaments could be seen. Some of these organisms retained a variable amount of Gram-positive stain in the end or in central portions. The subcultures only grew on 5% blood Columbia agar and on "chocolate" agar at 37°C in 10% CO_2, producing in 24 h, small (1 mm in diameter), grey, moist, round, raised, and non-hemolytic colonies. Identification of the Gram-negative bacillus isolated in the blood culture was rapidly performed by depositing a thin smear of this strain on a MALDI-steel plate. A saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid (1.1 μL) was applied to the bacterial smear and dried. Measurements were performed with a Microflex mass spectrometer (Bruker Daltonik, Wissembourg, France) using the FlexControl software (version 3.0). Mass spectra were acquired in a linear positive extraction mode ranging from 2,000 to 20,000 Da. The spectrum was imported into the BioTyper software (version 2.0; Bruker, Karlsruhe, Germany). The BioTyper database contains the spectra of approximately 3,739 species and is regularly updated by the Bruker Company. The result of the pattern-matching process was expressed with a score of 1.9, giving *Cardiobacterium hominis* DSM 8339T as first choice (a score of 1.9 was considered to be identification at species level [1]) (Fig. 1). Phenotypical identification using the VITEK 2 NH system (bioMérieux) performed on this oxidase-positive, catalase-negative, indole-producing strain indicated *C. hominis* (very good identification, with a probability of 93%). An in vitro susceptibility test, using a disk diffusion technique on Mueller-Hinton agar supplemented with 5% horse blood, showed that the organism was susceptible to ampicillin, amoxicillin plus clavulanic acid, piperacillin, cefotaxime, aminoglycosides, co-trimoxazole, vancomycin, and fluorinated-quinolones. The isolate was resistant to erythromycin. No β-lactamase was detected by the nitrocefin test.

To determine the involvement of *C. hominis* in the endocarditic process and to confirm its conventional

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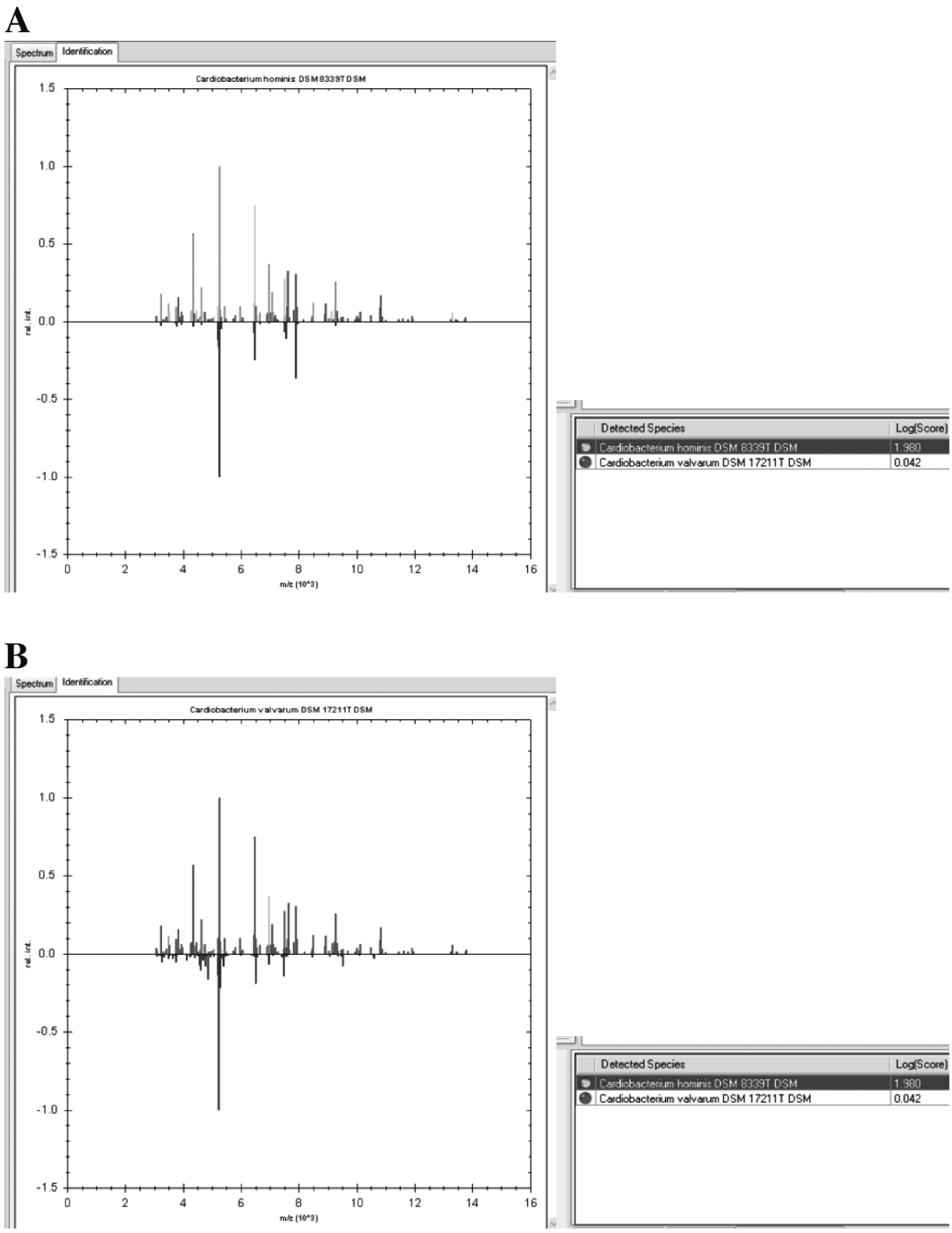

Fig. 1. (A) Spectrum of *C. hominis* (up) compared with the result of the database *C. hominis* DSM 8339T (down) with a score of 1.980. (B) Spectrum of *C. hominis* (up) compared with the result of the database *C. valvarum* DSM17211T (down) with a score of 0.042.

identification, the 16S rRNA gene was immediately sequenced from the colonies, and a further sample was obtained from a fragment of the valve preserved at −80°C. Using the primers described by Gauduchon et al. (2), a 16S rDNA bacterial fragment of 478 bp was amplified from the bacteria and sequenced on an automated sequencer (377 ABI Prism; PE Applied Biosystems, Foster City, Calif., USA). It was then compared with NCBI GenBank entries giving 98% (474/478 bp) identity with *C. hominis* strain 6573 (GenBank accession no. M35014). After DNA was extracted from the fragment of the aortic valve tissue (NucleoSpin ExtractII kit; Macherey Nagel, Hoerdt, France), amplification was performed using the same primers. After purification of the product using a SpinX kit (Costar, Cambridge, Mass., USA), the 470-bp fragment obtained was sequenced and compared with NCBI GenBank entries using the BLAST algorithm, giving 99% (468/470 bp) identity with *C. hominis* strain 6573 (GenBank accession no. M35014). From the two amplifications, the second species of *Cardiobacterium* (*C. valvarum*) showed 97% identity with *C. valvarum* strain MDA3079 (GenBank accession no. AF506987). This second species of *Cardiobacterium* was never mentioned

by the MALDI-TOF mass spectrometry knowing that this species belongs to the bank of the MALDI-TOF.

Aggregatibacter-Cardiobacterium-Eikenella-Kingella (ACEK) microorganisms are reported to cause 3% of all endocarditis cases. ACEK microorganisms frequently colonize the oropharynx, are slow growing, and their growth is enhanced by the presence of CO_2. *C. hominis* was isolated for the first time in 1962 from a patient with endocarditis and classified as a *Pasteurella*-like organism. In 1964, Slotnick and Dougherty described the organism and gave it the present name (3). *C. hominis* was recently reclassified with *Suttonella indologenes* in the *Cardiobacteriaceae* family on the basis of 16S rRNA sequence studies (4). Since the original publication, more than 90 cases of endocarditis have been documented (5–11). Because of the low virulence of the bacterium, *C. hominis* endocarditis is remarkably insidious in its presentation, with a tendency to infect damaged or prosthetic valves, which results in subacute or chronic endocarditis and the bacterium more frequently infects aortic valves than the other valves. Our case is new, only one case of this disease presenting as an acute apyretic coronary syndrome has been reported in the recent literature (10): the treatment with corticoids 15 days prior to the infection might have caused the beginning of this infectious illness.

Although it is recommended to increase blood culture incubation in order to detect *C. hominis*, improvements to conventional blood culture techniques have shown that *C. hominis* can be recovered from currently available automated blood culture systems within the standard incubation period of 5–6 days if the patient did not receive anti-microbial treatment, because *C. hominis* is very susceptible to antibiotics (12–14). In the last 15 years, the mean delay in obtaining positive blood cultures is 3.3 days in patients without anti-microbial treatment: only one case, described in 2006, required molecular techniques for rapidly obtaining the causal agent after 12 days of incubation (9). Finally, this case illustrates the ease and rapidity of MALDI-TOF mass spectrometry, which has good discrimination power to differentiate rapidly between the two species of *Cardiobacterium*, eliminating the need to perform molecular PCR (15–18). Nevertheless, this new technique does not propose additional biochemical or immunological tests if the MALDI-TOF result is not suitable (score <1.9). Improvements to conventional techniques (automated blood culture systems and MALDI-TOF mass spectrometry) and good coordination between the different care units enabled a rapid, documented diagnosis of *C. hominis* endocarditis. Moreover, an adapted therapy prevented the usual embolic complications often observed in this type of endocarditis (11).

Conflict of interest None to declare.

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