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Cardiac Device-Related Endocarditis Caused by Paenibacillus glucanolyticus

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On November 2012, a 65-year-old woman with type 2 diabetes was admitted to the University Hospital Center of Nancy because of a localized pacemaker pocket infection. The pacemaker was implanted 12 years before because of the patient’s high-degree atrioventricular block. The generator was replaced in 2010. Since January 2012, the patient had reported signs of inflammation at the site of pacemaker implantation. In October 2012, the patient, who was afebrile, was admitted to another hospital with local signs of inflammation at the generator pocket with skin fistulization and purulent discharge. Pocket abscess drainage was then performed, and a pus specimen was sent to the local laboratory. The patient, who was allergic to penicillin, was treated with trimethoprim-sulfamethoxazole and erythromycin for 10 days. Gram staining of the pus sample did not reveal any organism, while a rod-shaped bacterium that appeared to be Gram negative was obtained by culture. This isolate was identified first as Cronobacter sakazakii (Vitek 2 GN card; bioMérieux, Marcy l’Etoile, France) and finally as Aeromonas salmonicida (API ID 32GN kit; bioMérieux).

On admission to our hospital, the patient was afebrile, and physical examination revealed an inflammatory pacemaker pocket with a purulent discharge. No other symptoms were noted. The white blood cell count was 13.8 x 10^9/liter with 62% neutrophils. The serum C-reactive protein level was normal, while the serum procalcitonin level was slightly elevated. Transesophageal echocardiography revealed a hyperechogenic mass on the distal portion of the right atrial lead compatible with a vegetation. The patient underwent implantation of an epicardial pacemaker prior to percutaneous removal of the generator and the atrial and ventricular leads. The presence of a vegetation attached to the atrial lead was confirmed macroscopically. The patient was treated with ceftriaxone (2 g intravenously [i.v.] once a day) for 42 days. Four sets of blood cultures were drawn before the onset of antibiotic therapy. The leads as well as a pus sample obtained from the pacemaker pocket were sent for microbiological analysis.

Specimens taken during the intervention were processed using standard methods and inoculated onto tryptic soy agar with 5% sheep blood (bioMérieux) incubated at 37°C in air and brain heart infusion and Rosenow broths (Bio-Rad, Marnes-la-Coquette, France) were inoculated and incubated at 37°C, respectively, in air and anaerobically. Part of the vegetation embedding the atrial lead was also sampled for broad-range bacterial 16S rRNA gene PCR. Gram stains of the vegetation and the pus specimen obtained from the pocket did not reveal any organism. All blood cultures remained negative after 4 weeks of incubation (Bectec; Becton, Dickinson). After 24 to 48 h of incubation, smooth, flat, and white opaque colonies with spreading growth were observed on all plates (Fig. 1A and B). A Gram stain of the colonies revealed long and thin Gram-negative rods (Fig. 1C). Ellipsoidal and terminal spores swelling the sporangia became apparent after 48 to 72 h of incubation (Fig. 1D). Colonies from subcultures on Mueller-Hinton agar were catalase and oxidase positive. After 24 to 48 h, enrichment broths yielded the growth of the same organism. This isolate could not be identified by using the Vitek 2 Gram-positive identification card (GP card; bioMérieux). It was identified as Paenibacillus glucanolyticus (confidence value, 93.9%) on the basis of the biochemical profile obtained by the combined use of the API 50CH and API 20E kits (bioMérieux). This isolate was also identified as P. glucanolyticus (score, 2.201) by matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT device (Bruker Daltonics, Wissembourg, France) and the Biotyper v3.1 database (Bruker). At the time, the database contained 287 reference spectra for 17 genera and 196 species and subspecies of aerobically Gram-positive spore-forming rods (AGPSFR), including 101 Bacillus species (131 spectra) and 62 Paenibacillus species (112 spectra). This strain was also further tested with a Vitek MS system (bioMérieux). With this device, the isolate was identified as Paenibacillus sp. (confidence value, 99.99%) by using the MS-IVD v2.0 database and as P. glucanolyticus (Paenibacillus sp., 96.7%; supplementary details [compare mode], P. glucanolyticus, 74.6%) by using the Vitek MS RUO database. At the time, the MS-IVD database encompassed 8 genera and 46 species of AGPSFR, in-
including 19 *Bacillus* species (465 spectra) and 11 *Paenibacillus* species (132 spectra). The Vitek MS RUO database encompassed 8 genera and 49 species of AGPSFR, including 21 *Bacillus* species (427 spectra) and 13 *Paenibacillus* species (139 spectra). Species identification was confirmed by PCR amplification and sequencing of the 16S rRNA gene using the same primers as described previously (1). A consensus sequence of 589 bp was trimmed and submitted to the Ribosomal Database Project and GenBank for comparative analysis using the Basic Local Alignment Search Tool. The sequence of the 16S rRNA gene showed 99.66% homology with *P. glucanolyticus* NBRC 15330T (GenBank accession no. AB680838) and 99.32% homology with *P. glucanolyticus* DSM 5162T (GenBank accession no. NR_040883). The next highest homology (97.79%) was found for the 16S rRNA gene sequence of *Paenibacillus lautus* JCM 9073T (GenBank accession no. NR_040882). *P. glucanolyticus* was also detected in the vegetation by broad-range bacterial 16S rRNA gene PCR followed by sequencing. Antibiotic susceptibility was tested using Etest strips (bioMérieux). Non-species-related breakpoints established by the Antibiogram Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org) were used for interpretation. The isolate was intermediate to penicillin G and amoxicillin (MIC, 2 and 3 μg/ml, respectively), and susceptible to ceftriaxone (MIC, 0.5 μg/ml). β-Lactamase production was detected by the nitrocefin test (Cefinase disc; bioMérieux) (2). The isolate was found to be intermediate to ciprofloxacin (MIC, 0.94 μg/ml) and susceptible to erythromycin, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, vancomycin, rifampin, and linezolid (MICs, 0.19, 0.002/0.018, 0.64, 1, 0.5, 0.002, and 0.125 μg/ml, respectively).

Except for a postoperative course complicated by a noninfectious pericardial effusion that required surgical drainage 13 days after epicardial pacemaker implantation, the outcome was favorable and the patient was discharged after 30 days of hospitalization. Ceftriaxone was continued for another 2 weeks. No recurrence of infection was noted during the short-term follow-up.

Over the past few years, cardiac device-related infections, including endocarditis, have emerged as the consequence of an increased use of electrophysiological devices, especially in elderly and frail patients (3). Pacemaker-related endocarditis is due mainly to the migration of microorganisms from the implantation site to the distal intravascular part. Several factors, although under debate, have been associated with an increased risk of cardiac device infections, including device-related procedures, chronic renal failure, corticosteroid use, and diabetes (4–6). These infections may progress slowly and occur late after pacemaker implantation or last device manipulation (4). In our patient, diabetes mellitus could have been a contributing factor and the contamination might have occurred during the replacement of the generator 2.
years earlier, leading to the development of a late-onset infection. The diagnosis of lead-related endocarditis may be difficult, since patients with pacemaker infections may present without fever and blood cultures may be negative (6). These two features were observed in our case.

Device-related endocarditis is caused mainly by Staphylococcus spp., but other microorganisms that can be found on the skin and in the environment may be involved (6). So far, there have been only three reports of device-related endocarditis caused by Gram-positive endospore-forming rods (7–9). In all of these cases, a Bacillus sp. was involved. In our case, cardiac device-related infection was due to P. glucanolyticus, which has never so far been reported as a cause of human infection. Paenibacillus spp. are facultatively anaerobic or strictly aerobic, mostly motile endospore-forming Gram-variable rods that may be isolated from various environmental sources as well as from diseased insects and foods (10). Several Paenibacillus species have been reported as the cause of human infections, including bacteremia, endocarditis, meningitis, brain abscess, endophthalmitis, pneumonia, mediastinitis, prosthetic ostechoarticular infection, and chronic prostatitis (11–23). Paenibacillus glucanolyticus, formerly included in the genus Bacillus (24), is a facultatively anaerobic, long and thin rod-shaped bacterium that may be isolated from various soils (25). This organism appears mostly Gram negative and may produce oval spores that swell the sporangia (10). The Gram-negative appearance may be misleading for proper identification of these organisms, especially when spores have not become apparent. It is noteworthy that when we tested the P. glucanolyticus strain using a Vitek 2 Gram-negative identification card (GN card) and an API ID 32GN strip, results similar to those obtained with the apparently Gram-negative isolate found in October 2012 were observed. This suggests that the isolate obtained in October might have been misidentified. However, this remains a hypothesis, since the initial strain was no longer available for further characterization. P. glucanolyticus produces colonies that are flat, smooth, and white opaque and may show spreading growth. It is noticeable that the presence of motile colonies that rapidly migrate over agar media is a feature shared only by some taxa previously included in Paenibacillus spp. and Bacillus spp. The phenotypic characterization of paenibacilli may rely, as for other related endospore-forming species, on the combined use of API 20E and API 50CH kits (10). However, the precise identification of these bacteria to the species and even genus level may be difficult, considering the absence of clear-cut discriminating phenotypic characteristics and the fact that many proposals of new species have been made on the basis of the characterization of single isolates. Thus, molecular methods, including 16S rRNA gene sequence analysis, are often needed for the final identification of these organisms.

It has been shown that MALDI-TOF MS, which is increasingly used in clinical microbiology laboratories for the rapid identification of microorganisms, performed as well as extended phenotypic methods and specific molecular methods to identify aerobic Gram-positive rods, including Bacillus spp. and Paenibacillus spp. (26, 27). In our case, the P. glucanolyticus isolate, which could not be identified by using the Vitek 2 GP card since the Paenibacillus genus is not included in the database, was correctly identified using either the API 50CH and API 20E strips, MALDI-TOF MS, or 16 S rRNA gene sequence analysis. The two major commercial MALDI-TOF MS systems are the Bruker Biotyper and the bioMérieux Vitek MS systems (28). The databases of both systems contain mass spectra of P. glucanolyticus. SuperSpectra, which are consensus spectra that are typical at a defined taxonomic level, are used in the Vitek MS RUO system during the fully automated first-line identification step. Our isolate could not be identified beyond the genus level using this approach since no SuperSpectrum specific for P. glucanolyticus was, at the time, included in the RUO database. With the Vitek MS IVD system, the isolate was also only identified to the genus level using the Advanced Spectra Classifier approach, which represents an extension of the SuperSpectrum approach (28). Our isolate could be identified beyond the genus level with the Bruker Biotyper system and the Vitek MS RUO system when the compare mode was used. The latter approach represents a second-line identification method based, as is the case for the Biotyper system, on the direct comparison of the isolate spectrum to all reference spectra of the database. This result highlights the fact that results obtained with MALDI-TOF MS systems depend not only on the database contents but also on the algorithms used for interpretation of crude data.

This is, to the best of our knowledge, the first report showing that P. glucanolyticus may be a pathogen in humans and that a member of the genus Paenibacillus may be involved in device-related endocarditis. This case also highlights the fact that the routine use of mass spectrometry is a rapid and accurate method that may be used for the identification of Gram-positive aerobic rods, such as Paenibacillus, for which the Gram staining reaction may be misleading.

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