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Misidentification of *Mycobacterium peregrinum*, the Causal Organism of a Case of Bacteremia and Automatic Implantable Cardioverter Defibrillator-Associated Infection, Due to Its Unusual Acid-Fast Staining Characteristics

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We report an unusual case of *Mycobacterium peregrinum* bacteremia and infection of an automatic implantable cardioverter defibrillator that was originally misidentified as a *Nocardia* sp. due, in part, to its partially acid-fast staining characteristic, morphology, and odor. The misdiagnosis had a direct effect on patient care, though the patient was subsequently successfully treated.

CASE REPORT

A 74-year-old white male was admitted with a past history of ischemic cardiomyopathy, coronary artery disease, hypertension, and a recent diagnosis of nonsustained ventricular tachycardia requiring placement of an automatic implantable cardioverter defibrillator (AICD) 6 weeks earlier. He presented to our institution with persistent erythema and a history of a pustule around the AICD insertion site on his left chest wall. The patient stated he immediately noted erythema around the insertion site after AICD placement and was prescribed a 10-day course of cephalexin at a 3-week follow-up with his cardiologist. Subsequently, he noted a pustule along the suture line and was admitted for further evaluation and management. On physical examination he was nontoxic in appearance and afebrile, his vital signs were stable, and his physical examination was within normal limits except for the left chest area. The left chest wall was erythematous in an area of 4 to 5 cm where the AICD site had been inserted subcutaneously; there was a deep purple discoloration along the incision line, and the area was warm to touch and minimally tender to palpation. There was no obvious pustule or purulent discharge along the incision, and the surrounding area was not fluctuant. Laboratory data were unremarkable except for a creatinine level of 1.7 mg/dl, which was his baseline. Blood cultures were drawn on admission, and empirical vancomycin was begun. On the day of admission he underwent extraction of the AICD and the leads in the electrophysiology laboratory. Deep-pocket tissue and superficial wound specimens were sent for routine bacterial, fungal, and acid-fast bacillus cultures.

The described wound specimens and two sets of blood cultures were submitted to the microbiology laboratory for bacterial culture. Direct Gram-stained smears of the wound cultures demonstrated rare to few polymorphonuclear leukocytes with no organisms seen. After 4 days of incubation, growth was detected in both blood cultures and in the thioglycolate broth media of both wound cultures.

On the fifth hospital day, Gram-stained smears of the wound broth cultures were reported as having gram-positive rods. One day later, growth was detected on the sheep blood and chocolate agar plates, and no growth was detected on the MacConkey agar plate. Colonies were tan, flat, and dry and upon further incubation became wrinkled in appearance and exhibited a musty odor. The Vitek Identification System (bioMérieux, Hazelwood, MO) was unable to identify the isolate. The isolate was subcultured to Columbia agar for identification using the API CORYNE system (bioMérieux, Hazelwood, MO), but it too yielded no identification. A modified acid-fast (MAF) stain of the isolate using an aqueous decolorization agent of 1% sulfuric acid demonstrated a partially acid-fast organism. Specifically it revealed blue organisms stained only with counterstain and red organisms that remained acid fast, some of which had occasional filamentous branching.

Additionally, the Gram-stained smear of the blood culture isolates also revealed gram-positive rods, and these were subcultured to sheep blood, chocolate, and MacConkey agar plates. After 3 days of incubation, colonies were detected on the sheep blood and chocolate agar plates, and no growth was detected on the MacConkey agar plate. Colony morphology was identical to the wound culture isolates. A Gram stain also demonstrated gram-positive rods. On the eighth hospital day the organism was identified as a presumptive *Nocardia* species given the Gram stain appearance, MAF stain results, colonial morphology, and odor. One of the blood culture isolates was sent for species-level identification to an affiliate hospital laboratory (Hahnemann University Hospital, Philadelphia, PA) and was subcultured to sheep blood and chocolate agar plates.

Based upon the microbiology report, as well as discussions between clinicians and the microbiologists, several clinical decisions were made. First, the patient's regimen was changed to

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trimethoprim-sulfamethoxazole (TMP-SMZ), the drug of choice for suspected nocardial infections. Second the patient underwent computerized axial topography scans with intravenous contrast of both the chest and brain to rule out the possibility of disseminated nocardial infection, given the well-described nature and clinical course of such infections. Both were negative for any suspicious infectious lesions. Third, additional blood cultures were drawn. Fourth, the patient's hospital course was extended by several days to complete the additional testing and to monitor the change in antimicrobial therapy. He was discharged on the 11th hospital day to home.

Within a day of the patient's discharge on TMP-SMZ, the affiliate hospital laboratory reported the organism might not be a *Nocardia* sp., suggested other organisms should be considered, and stated further identification was in progress. Specifically, upon review of a Gram stain of the blood isolate, the differential was expanded to include mycobacterial pathogens and other aerobic actinomycoses, as well as *Tsukamurella* spp., in part due to the clinical picture and overlapping laboratory findings (11). Three days later, the patient developed acute renal insufficiency, due to the TMP-SMZ, and was rehospitalized for further management of his renal disease. All antimicrobial therapy was discontinued pending identification of the isolate. The subsequent blood cultures obtained after removal of the AICD were negative. After a 5-day hospital stay, the patient's renal function returned to baseline and he was again discharged to home, this time off all antimicrobial therapy.

In the laboratory, after two days of incubation, subcultured growth of the isolate was used to inoculate a lysozyme test; a quad plate containing casein, tyrosine, xanthine, and starch agars; and a Löwenstein-Jensen slant. The isolate subsequently tested lysozyme sensitive, suggesting it was a *Mycobacterium* sp. and not a *Nocardia* sp. or *Tsukamurella* sp., both of which are routinely lysozyme resistant. A Kinyoun acid-fast stain (cold procedure) and MAF stain were then performed. The isolate was positive on both acid-fast stains. Because of the lysozyme and staining results and the failure of the isolate to hydrolyze amino acids or starch in the quad plate, biochemicals for the identification of *Mycobacterium* spp. were then inoculated. The isolate was sent to a second reference laboratory (Indiana University, Indianapolis, IN) for rapid identification by mycolic acid analysis using high-pressure liquid chromatography. This confirmed the isolate was a member of the rapidly growing, atypical mycobacterium *Mycobacterium fortuitum* complex. Biochemical results confirmed the identification. Specifically, the isolate grew on MacConkey agar without crystal violet at room temperature and was positive for nitrate reduction, urea hydrolysis, and arylsulfatase activity at 3 days and for tellurite reduction and was negative for Tween 80 hydrolysis. For complete definitive identification and susceptibility testing, the isolate was sent to a third reference laboratory (University of Texas Health Center, Tyler, TX). Identification by PCR, as previously described, confirmed that the isolate was *Mycobacterium peregrinum* (10). Susceptibility testing by the Kirby-Bauer method revealed sensitivity to amikacin, ciprofloxacin, clarithromycin, doxycycline, gatifloxacin, imipenem, linezolid, meropenem, and tobramycin. Subsequent subcultures of the isolate on chocolate agar revealed two colonial morphotypes: a flat, dry, and wrinkled type and a small, moist, domed colony. Both colonial morphotypes of the subcultured isolate exhibited enhanced staining with both the modified Kinyoun and Kinyoun acid-fast stains.

At his outpatient follow-up visit 2 weeks after his second discharge, the patient was well but still had some erythema and violaceous discoloration of the left chest wall at the previous AICD site. Purulent discharge could be expressed and was again cultured. Three days prior to this office visit, the organism had been identified as a member of the *Mycobacterium fortuitum* complex. The patient was therefore prescribed an oral regimen of ciprofloxacin and clarithromycin. A new AICD was implanted 4 weeks into dual antibiotic therapy. Despite recommendations for a 3-month antibiotic regimen, the patient chose not to take further antibiotic therapy after completing 6 weeks. The culture obtained at the time of the outpatient follow-up visit did grow *M. peregrinum*; however, the patient remained asymptomatic, the left chest wound completely healed, and the new AICD site remained unaffected.

Conclusions. There are an estimated 3.25 million patients with functioning pacemakers and 180,000 patients with AICDs in the United States (4, 6). Rates of infection for these implantable foreign bodies range from 1 to 7% (4, 6). The majority are caused by skin flora, with *Staphylococcus aureus* and coagulase-negative staphylococci accounting for 63 to 75% (6). Streptococci, corynebacteria, propionibacteria, and *Enterobacteriaceae* members make up a smaller percentage (6). To our knowledge this is the first reported case of a rapid-growing atypical mycobacterium, specifically *M. peregrinum*, as the causative agent in such an infection. There have been two recently published cases of *M. peregrinum* infection of a central line and a Hickman catheter (5, 9).

The Runyon group IV rapidly growing mycobacteria produce visible growth on solid media within 7 days (3). When Gram stained, rapidly growing mycobacteria may appear as gram-positive bacilli and can be mistaken for *Corynebacterium* species, diphtheroids, or *Nocardia* spp., if further testing is not performed (3). Those implicated in disease in humans are the *Mycobacterium fortuitum* group, the *Mycobacterium chelonae*/*abscessus* group, and the *Mycobacterium smegmatis* group (3). The *Mycobacterium fortuitum* group can be further subdivided into *Mycobacterium fortuitum*, *Mycobacterium peregrinum*, and a third unnamed complex (3). These organisms are ubiquitous in nature and can be found in soil and water. They may occasionally be found as commensal organisms on human skin (8). It has been postulated that these organisms may preferentially colonize the anterior chest wall, since the majority of documented human infections involve the anterior chest such as tunneled catheter infections, sternotomy wound infections, and infections after augmentation mammoplasty procedures (7, 8).

In the case presented, confusion concerning the identification of the clinical isolate, *Mycobacterium peregrinum*, occurred because the isolate initially stained as partially acid fast. This unusual feature, combined with its relatively rapid growth in culture, led to a presumptive misidentification of *Nocardia* spp., which adversely affected patient care.

Uncertainty often exists in the clinical microbiology laboratory regarding isolates that demonstrate general characteristics

of nocardioform organisms. Clinical isolates of nocardiae may be presumptively identified based on biochemical tests; microscopic appearance, including MAF staining of direct smears or culture isolates; and macroscopic morphology (1, 2). Usually nocardiae, along with *Gordona*, *Dietzia*, *Rhodococcus*, and *Tsukamurella* spp., stain partially acid fast; that is, they exhibit both acid-fast and non-acid-fast bacilli and filaments in a single MAF stain (1, 2). However, nocardiae may on occasion stain MAF negative or stain strongly MAF positive, thus resembling acid-fast mycobacteria. In the latter case, isolates can be differentiated from mycobacteria using pyridine extraction prior to Kinyoun acid-fast staining (1, 2). In contrast, *Mycobacteria* spp., which possess large and complex mycolic acids, stain strongly acid fast with both the MAF and Kinyoun acid-fast stains (3). In the present case, however, the *M. peregrinum* isolate exhibited only a partially acid-fast staining pattern when first grown, thus leading both the clinical microbiologists and physicians to believe it to be a possible *Nocardia* sp.

Nocardia spp. isolated in culture often demonstrate branching hyphae, which may later fragment into coccoid and bacillary elements (2). However, they may appear in various morphological forms under the influence of many factors including medium composition, incubation temperature, and the duration of the incubation (1). Macroscopically, colonies of *Nocardia* and *Mycobacterium* spp. can look the same (1). Both groups often appear as dry, flat, wrinkled colonies, thus furthering the potential for confusion if additional identification procedures are not employed.

Our case highlights that both *Nocardia* spp. and *M. peregrinum* can demonstrate overlapping MAF staining and morphological patterns microscopically, as well as common colonial and cultural characteristics, which could lead to misidentification and diagnostic confusion. The original misdiagnosis of the isolate as a *Nocardia* sp. led to a series of clinical decisions, including prescription of a different antimicrobial and administration of intravenous contrast for computed tomography scanning. Both of these likely contributed to the patient's subsequent acute renal insufficiency and need for repeat hospitalization. Fortunately there were no permanent sequelae; however, there were significant additional healthcare costs accrued and there was an increase in morbidity for the patient. With respect to reporting, a presumptive distinction between *Nocardia* spp. and mycobacteria should always be based upon the Gram stain, MAF stain, and Kinyoun acid-fast stain, with additional data such as demonstration of aerial hyphae and phenotypic test results for lysozyme and arylsulfatase activity, if available. Colonial morphology and odor, while often helpful, can also be misleading and therefore should not be used in lieu of the above-named tests. In particular, such diagnostic approaches are critical for those isolates that do not exhibit obvious filamentous branching. In addition, reference laboratories specializing in mycobacterial and aerobic actinomycete identification should be fully utilized for isolates lacking usual morphological and or phenotypic characteristics. Reference testing at these sites includes chemotaxonomic investigations like cellular fatty acid analysis, mycolic acid analysis, and cell wall analysis and molecular analysis such as 16S rRNA gene sequencing, G + C content, and DNA-DNA hybridization. These methodologies are beyond the scope and capabilities of most clinical microbiology laboratories. In conclusion the first reported case of *M. peregrinum* bacteremia secondary to AICD infection posed several clinical and laboratory challenges, and in the future it should be recognized that this organism may on rare occasions exhibit atypical acid-fast staining patterns.

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