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First Report of Human Infection by *Agromyces mediolanus*, a Gram-Positive Organism Found in Soil

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We report the first human infection by a member of the *Agromyces* genus, a group of Gram-positive bacteria found in soil. A patient with a long-term venous catheter developed bacteremia due to a non-vancomycin-susceptible isolate of *Agromyces mediolanus*. Rapid identification was possible by matrix-assisted laser desorption ionization–time of flight mass spectrometry.

CASE REPORT

A 95-year-old man with a history of bronchiectasis was admitted to hospital due to acute bronchiectatic exacerbation and pneumonia. He required prolonged (>6 months) inpatient care for pulmonary rehabilitation and nutritional support. In addition to percutaneous gastrostomy tube feeding, he also received parenteral nutrition during the 6 months of hospitalization. Venous access was achieved with a double-lumen peripherally inserted central catheter (PICC) that had been inserted into the left brachial vein 4 months previously.

He developed a fever of 38.5°C on day 150 of hospitalization, after all antibiotics had been discontinued for 2 weeks. His sputum volume was static, and a chest X-ray did not show any interval changes. The percutaneous gastrostomy exit site was clean. The PICC line exit site was not erythematous; however, resistance was encountered when obtaining blood cultures from the PICC lumens, indicating blockage. His total white blood cell count was 3.0×10^9/liter (reference range, 4.5×10^9 to 11.0×10^9/liter), his neutrophil count was 2.20×10^9/liter (reference range, 2.0×10^9 to 7.0×10^9/liter), and his lymphocyte count was 0.40×10^9/liter (reference range, 1.0×10^9 to 3.0×10^9/liter). His alkaline phosphatase level was marginally elevated at 128 U/liter (reference range, 47 to 123 U/liter), while his other liver function test parameters were normal.

Three pairs of blood cultures were obtained from a peripheral vein and both PICC lumens. As he was otherwise clinically well with no identifiable focus of infection, antibiotics were not started at this stage. Subsequently, all aerobic blood cultures turned positive, yielding Gram-positive rods. In addition, one of the three blood cultures obtained via the PICC lumen yielded extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* simultaneously with the Gram-positive rod. Once the initial blood cultures turned positive, cultures of blood from both PICC lumens and a peripheral vein were immediately repeated (24 h after the first set of cultures was obtained), after which he was started on intravenous meropenem at 500 mg every 6 h and intravenous vancomycin at 500 mg every 8 h. All three sets of repeat blood cultures yielded Gram-positive rods again, but none grew *E. coli*.

All of the six pairs of aerobic and anaerobic blood culture bottles (Bactec Plus Aerobic/F, Bactec Lytic/Anaerobic/F; Becton, Dickinson) used to test blood obtained from this patient were incubated in the Bactec blood culture system (Becton, Dickinson). All of the aerobic bottles yielded irregular coryneform Gram-positive rods after 24 to 36 h of incubation. Following overnight incubation at 37°C in a 5% CO_2 environment, nonpigmented, nonhemolytic colonies were observed on 5% horse blood agar. No growth was observed on MacConkey agar. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was performed with a MALDI Biotyper 2.3 (Bruker Corporation, Billerica, MA) (1, 2) by the direct transfer method, and all of the isolates were identified as *Agromyces mediolanus* with scores ranging between 2.12 and 2.47 (the threshold score for confident identification with the Bruker MALDI Biotyper system is 2). The database contained three reference spectra for *A. mediolanus* and also contained spectra for 12 other *Agromyces* species, enabling confident identification to the species level.

On the basis of the 10-h difference in the time to positivity between the PICC lumen blood cultures and the peripheral blood culture, a catheter-related bloodstream infection was suspected and the PICC line was removed. The catheter tip yielded a significant count of methicillin-resistant coagulase-negative staphylococci but not *A. mediolanus*. A transthoracic echocardiogram did not reveal any vegetation.

Although blood cultures no longer yielded any microorganisms after commencement of antibiotics, the patient had a persistent fever even 3 days after line removal. On the basis of the results of antimicrobial susceptibility testing of the Gram-positive rods (Table 1), meropenem was switched to intravenous imipenem-cilastatin at 500 mg every 8 h. Vancomycin was discontinued 3 days after line removal. The patient defervesced rapidly on imipenem-cilastatin and completed a 14-day course of treatment. However, shortly after cessation of antibiotics, he developed recurrent *E. coli* bacteremia (a non-ESBL-producing strain with an

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TABLE 1 Etest MICs of various antibiotics tested against patient isolate AM01

Antibiotic	MIC (μg/ml)
Penicillin	2.0
Ceftriaxone	8.0
Meropenem	3.0
Imipenem	0.5
Vancomycin	48

antibiogram different from that of the isolate from the first episode). Imaging of the abdomen for an underlying infective focus by positron emission tomography-computed tomography scan revealed a colonic tumor.

16S rRNA gene sequencing. The first patient isolate (AM01) was subjected to DNA extraction, partial 16S rRNA gene PCR amplification with primers 1F 5′-AGTTTGATCMTGGCTCAG-3′ and 2R 5′-GGACTACHAGGGTATCTAAT-3′, and sequencing as described in our previous publications for aerobic Gram-positive rods (2–7). The sequences were compared to 16S rRNA gene sequences in the GenBank database by multiple-sequence alignment with Clustal W (8). Phylogenetic relationships were determined by the maximum-likelihood method (Fig. 1). A total of 679 nucleotide positions were included in the analysis. There were three base differences between the amplified sequence of AM01 and a partial sequence of the 16S rRNA gene of *A. mediolanus* reference strain JCM 3346 (GenBank accession no. NR_117879), corresponding to a nucleotide match of 99.6%. On the basis of the results of MALDI-TOF MS and 16S rRNA gene sequencing, AM01 was identified as *A. mediolanus*.

Antimicrobial susceptibility testing. The MICs of penicillin, ceftriaxone, meropenem, imipenem, and vancomycin for AM01 were determined by Etest (bioMérieux, Marcy-l'Étoile, France) on Mueller-Hinton agar with blood. The results are presented in Table 1. In addition, disk diffusion tests were performed with clindamycin and erythromycin on Mueller-Hinton agar with blood. Both Etest and disk diffusion results were read at 24 h of incubation. Vancomycin and ceftriaxone had the highest MICs (48 and 8 μg/ml, respectively), while clindamycin and erythromycin disk diffusion testing showed no inhibition zone, indicating resistance to these agents. Imipenem had the lowest MIC (0.5 μg/ml) of all of the antibiotics tested. Meropenem, one of the empirical antibiotics used in this patient, had a higher MIC of 3 μg/ml. Although there are no interpretative MIC breakpoints for this organism, we believe that the prompt clinical response to imipenem-cilastatin combined with its low MIC makes this antibiotic a more suitable choice than meropenem for treating infections due to *A. mediolanus*.

We describe the first case of human infection with *A. mediolanus*, a catalase-positive Gram-positive rod that was first assigned to the *Agromyces* genus in 1996 (9). The *Agromyces* genus (family *Microbacteriaceae*) comprises an expanding group of environmental saprophytes found in soil and plant rhizosphere samples. The human pathogens most closely related to *Agromyces* belong to the *Microbacterium* genus, which are rare causes of opportunistic infections in immunocompromised patient.

The isolation of *A. mediolanus* from the bloodstream of this patient is likely to be highly significant. First, all six of the blood cultures obtained before commencement of antibiotics yielded *A. mediolanus* but only one yielded *E. coli*. Second, of the three blood cultures obtained on the 2nd day before starting antibiotics, none yielded *E. coli* but *A. mediolanus* was persistently isolated from the patient, who remained febrile. Third, the patient's fever persisted despite meropenem, an effective regimen for *E. coli*, and line removal and subsided only after imipenem-cilastatin, which showed better *in vitro* activity against the *A. mediolanus* isolate, was started. Persistent isolation of *A. mediolanus* from the blood cultures of a febrile patient and his response only to specific anti-

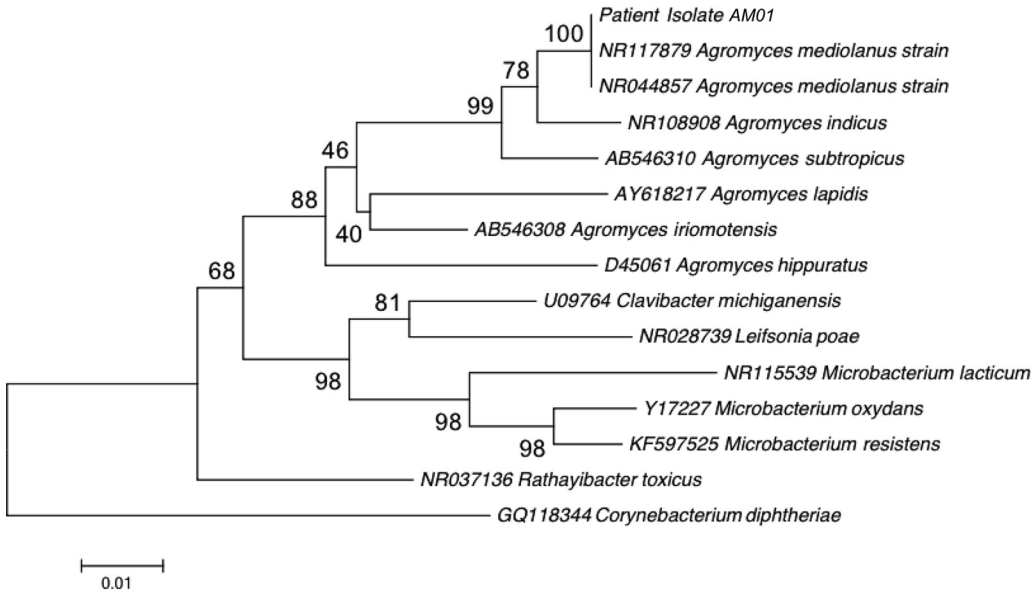

FIG 1 Phylogenetic analysis of the 16S rRNA gene sequences of isolate AM01 and other related species. The tree shown was constructed by the neighbor-joining method with 1,000 bootstrap replicates. The scale bar indicates the estimated number of nucleotide substitutions per 50 nucleotides. Names and accession numbers are shown as cited in the GenBank database.

biotic treatment active against *A. mediolanus* are factors that strongly favor *A. mediolanus* as being the major pathogen in this patient. Although *E. coli* bacteremia recurred shortly after cessation of antibiotics, it is notable that the isolate found on recurrence did not produce an ESBL and was sensitive to cefuroxime, unlike the first *E. coli* isolate. This excludes persistence of ESBL-producing *E. coli* in the bloodstream as a cause of his fever.

The exact source of *A. mediolanus* bacteremia in our patient remained ambiguous, but there are several possibilities. On the basis of the differential time to positivity of the blood cultures obtained from the PICC lumens and a peripheral vein, a catheter-associated bloodstream infection was originally suspected. However, we were unable to isolate the organism from the explanted catheter tip. Another possibility was contamination of the parenteral nutrition infusate. However, the bag had already been discarded and was not available for testing. The coisolation of *E. coli* in one blood culture and the subsequent diagnosis of a colonic tumor also raised the possibility of translocation of *A. mediolanus* from the gut. Although *Agromyces* species have not been detected in human gut microbiome studies, members of this genus have recently been detected in foods such as freshwater fish (10), raising the possibility of temporary food-borne human gut colonization. The subsequent recurrence of *E. coli* bacteremia even after removal and exchange of the PICC supported the hypothesis that the colonic lesion was the likely portal of entry, but a catheter-related infection could not be excluded.

The identification of rarely encountered pathogens can pose significant challenges to the clinical microbiology laboratory. However, in this case, rapid laboratory identification of the agent was made possible by MALDI-TOF MS and this result confirmed the utility of this method for the identification of rarely encountered bacteria when reference spectra are represented in databases (1).

The most striking feature of the sensitivity test results was the organism's high-level resistance to vancomycin, a narrow-spectrum antibiotic that is active against most Gram-positive organisms (11). This also corresponded to the patient's poor clinical response, as he remained febrile despite treatment with intravenous vancomycin and catheter removal. This might be due to the unusual amino acid composition of the cell wall peptidoglycan of members of the *Agromyces* genus, namely, the abundance of 2,4-diaminobutyric acid (2,4-DAB) in the peptide subunits and cross-links (12), which might hinder the binding and activity of glycopeptide antibiotics like vancomycin. Further studies on the antibiotic resistance profiles of bacteria with abundant 2,4-DAB in their cell walls (such as members of the *Agromyces*, *Clavibacter*, and *Rathayibacter* genera) are required to confirm this interesting observation. Macrolide and lincosamide resistance and a high ceftriaxone MIC further limited the choice of antibiotics for treating the infection. The patient remained febrile while on meropenem but promptly defervesced after imipenem-cilastatin was started, which corresponded to the lower Etest MIC of the latter antibiotic. Vancomycin is a commonly used effective empirical antibiotic for patients with suspected Gram-positive infections, but *A. mediolanus* appears to be an exception to this rule and may be included in the list of Gram-positive rods that are resistant to vancomycin, a list that includes *Leuconostoc*, *Pediococcus*, *Lactobacillus* species, and *Erysipelothrix rhusiopathiae*. Therefore, isolation of *A. mediolanus* should prompt clinicians to switch to an alternative like imipenem-cilastatin, which was effective in our patient.

In summary, we report here the first documented case of human infection by *A. mediolanus*, manifesting itself as bacteremia in an elderly patient with a long-term venous catheter and an underlying colonic tumor. The isolate was readily identified by MALDI-TOF MS and 16S rRNA gene sequencing. The organism demonstrated resistance to multiple antibiotics, including vancomycin. Imipenem-cilastatin appeared to be active *in vitro* and *in vivo*.

Nucleotide sequence accession number. The 16S rRNA gene sequence of isolate AM01 has been deposited in the GenBank database under accession number KR780769.

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We have no conflict of interest to declare.

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