

## Bacteremia Caused by an Undescribed Species of *Janibacter*

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**A yellow-pigmented rod- to coccoid-shaped coryneform microorganism was isolated from the blood of a patient with acute myeloid leukemia. It was identified by 16S rRNA gene sequencing as a previously undescribed species of *Janibacter*. The isolate was susceptible to penicillins, aminoglycosides, fluoroquinolones, and glycopeptides.**

### CASE REPORT

In October 2001, an acute myeloid leukemia was diagnosed in a 51-year-old man. Karyotype analysis confirmed the presence of the Philadelphia chromosome, resulting from a reciprocal translocation between chromosomes 9 and 22 that leads to the production of an abnormal fusion protein (b2a2) with tyrosine kinase activity. The patient was treated with oral hydroxyurea (50 mg/kg of body weight/day for 3 days) followed by a combination of intravenous cytarabine (200 mg/m<sup>2</sup>/day for 7 days) and intravenous idarubicin (8 mg/m<sup>2</sup>/day for 5 days). When complete remission was achieved, as shown by the normalization of blood cell counts and karyotype, the patient was treated with imatinib (400 mg/day per os), a specific inhibitor of the abnormal tyrosine kinase protein. On 15 January 2002, the patient was admitted at the University Hospital of Hôtel-Dieu (Paris, France) to undergo an allogeneic hematopoietic stem cell transplantation. On day 0 (D0; hereafter, all days are designated with reference to D0, with days before D0 being assigned negative values [e.g., D-9 is 9 days before D0] and days after D0 being assigned positive values [e.g., D+3 is 3 days after D0]), 8 February 2002, bone marrow cells were collected from his full-matched HLA-identical brother and infused. Before the transplantation, a myeloablative conditioning regimen consisting of oral busulfan (1 mg/kg/6 h from D-9 to D-6) in combination with cyclophosphamide (60 mg/kg/day from D-4 to D-3) through a central venous catheter was administered. The catheter had been introduced in January and kept in place for the following weeks. Graft-versus-host disease (GVHD) prophylaxis consisted of intravenous first and then oral cyclosporine (4 mg/kg/day from D-1 to D+180) in combination with intravenous methotrexate (15 mg/m<sup>2</sup> at D+1 and 10 mg/m<sup>2</sup> at D+3 and D+6). Prevention of veno-occlusive disease was obtained with intravenous heparin (100 IU/kg/day). To limit the risk of bacterial infection, the patient underwent a gut decontamination with oral antibiotics (colistin

and gentamicin) and a daily skin disinfection. The patient was hospitalized in a laminar airflow room until recovery from severe aplasia. During the aplastic period (D0 to D+20), the patient developed grade 4 mucositis that needed morphine analgesia. On D+7, the patient presented with fever (38.5°C) that led to an antibiotherapy consisting of piperacillin-tazobactam (200 mg/kg/day) and tobramycin (4 mg/kg/day) for 14 days. The fever subsided in 24 h. The hematopoietic recovery started at D+18, as shown by the increase of white blood cell count (10<sup>3</sup> cells per  $\mu$ l), and was obtained at D+20 (0.5  $\times$  10<sup>3</sup> neutrophils per  $\mu$ l and 50  $\times$  10<sup>3</sup> platelets per  $\mu$ l). Thus, on D+20, the patient left the sterile room. Neither acute nor chronic GVHD were seen. A complete chimerism was obtained, as shown by the total replacement of the patient's bone marrow cells by his brother's cells. Cultures from blood samples taken both from the catheter and from a peripheral vein were performed. Interestingly, a strain of a gram-positive bacillus was isolated from five blood samples, including one taken from a peripheral vein at D-1 and four from the catheter at D+3, D+6, D+10, and D+13. Bacterial cultures of the catheter, removed at D+27, remained negative. At present (December 2004), the patient is in complete remission, and his Karnofsky score performance status is 100%.

Blood samples were inoculated in aerobic and anaerobic blood culture vials (BACTEC PLUS AEROBIC/F and PLUS ANAEROBIC/F; BD Diagnostic Systems, Sparks, Md.). The gram-positive bacillus was isolated from aerobic vials that were subcultured on nutrient agar. After 24 h of incubation at 37°C, colonies were cream-colored or yellowish, circular, opaque, glistening, and convex with entire margins. Staining of the bacteria at the early stage of growth showed gram-positive bacilli that occur singly, in pairs, or in irregular clumps. In older cultures, the shape of cells changed from short rods to coccoid cells. The microorganism (isolate 03-238) was nonmotile, non-spore-forming, strictly aerobic, catalase positive, and oxidase negative. Gelatin was hydrolyzed. No acid was produced from carbohydrates. Nitrate was not reduced to nitrite. Urease was not produced. Indole was not produced from tryptophan. In an attempt to identify this isolate, API Coryne strip (bioMérieux, Marcy l'Etoile, France) was used as recommended by the manufacturer. The repeated

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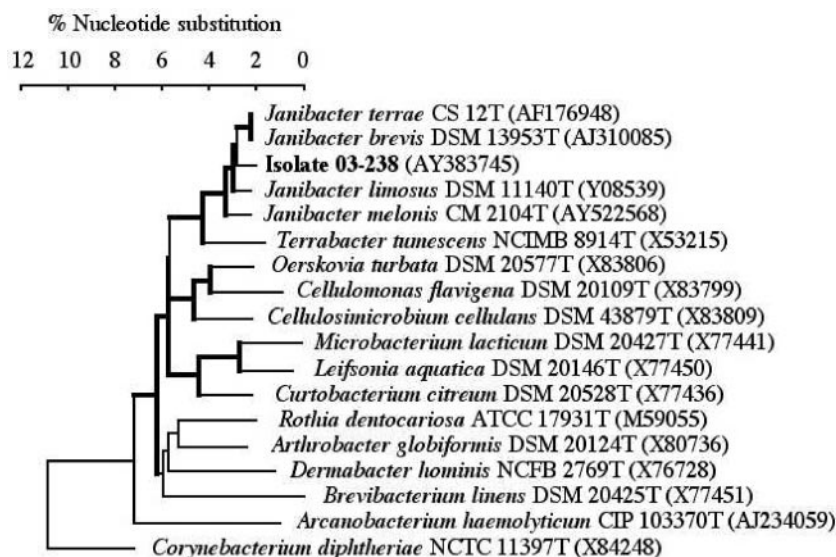


FIG. 1. Phylogenetic tree of isolate 03-238 of *Janibacter* sp. and the type strains of related coryneform species based on comparative analysis of 16S rRNA gene sequences. For genera other than *Janibacter*, only type species were included. Sequence alignment and tree construction were with ClustalV and by use of MegAlign, a program of the Lasergene package (DNASTAR). Clusters with bootstrap values above 80% are indicated with thick lines. Other values were below 52%.

bacterial identification obtained with API Coryne strip was *Arthrobacter* sp. (code no. 2112004; percentage of identification of 37.5%; index of typicality of 0.95). Positive results were for pyrazinamidase, alkaline phosphatase,  $\alpha$ -glucosidase, and gelatin hydrolysis. These results prompted us to determine the 16S rRNA gene sequence of the isolate as previously described (3). Briefly, the 16S rRNA gene was amplified by PCR with the primers Al (5'-AGRGTTYGATYCTGGCTCAGGAYG-3') and rJ (5'-GGTTACCTTGTTACGACTT-3') (5). A total of 1,405 continuous nucleotides were determined. The complete 16S rRNA gene sequence of the isolate was compared to all bacterial sequences available from the GenBank database by using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/BLAST.cgi>) and showed 98% similarity to each of the sequences of the type strains of *Janibacter brevis* (GenBank accession no. AJ310085), *Janibacter terrae* (GenBank accession no. AF176948), and *Janibacter limosus* (GenBank accession no. Y08539). It also showed 97% similarity to the sequence of the type strain of *Janibacter melonis* (GenBank accession no. AY522568). These five 16S rRNA gene sequences were aligned with ClustalV, and a phylogenetic tree was constructed by using MegAlign, a program of the Lasergene package (DNASTAR, Madison, WI). Moreover, 13 type species of related genera were included in this phylogenetic study. Bootstrap analysis (1,000 resamplings) was performed with the use of PAUP software (9) (Fig. 1). Antimicrobial susceptibility of the isolate was determined by the agar diffusion method using the Epsilon meter test (E test; AB Biodisk, Solna, Sweden) on Mueller-Hinton agar as recommended by the manufacturer and according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (<http://www.sfm.asso.fr>) (8). MIC results were as follows: penicillin G, 0.5  $\mu$ g/ml; amoxicillin, 0.38  $\mu$ g/ml; piperacillin, 0.38  $\mu$ g/ml; cefotaxime, 4  $\mu$ g/ml; cefepime, 8  $\mu$ g/ml; gentamicin, 0.75  $\mu$ g/ml; tobramycin,

0.5  $\mu$ g/ml; ciprofloxacin, 0.25  $\mu$ g/ml; vancomycin, 0.38  $\mu$ g/ml; and teicoplanin, 0.25  $\mu$ g/ml.

The genus *Janibacter* has been recently proposed with the description of *J. limosus*, isolated from sludge (7). Janus, a god in Roman mythology, is said to have had two faces, and thus the name *Janibacter* refers to the changing morphology of the microorganisms during growth. It should be noted that other related genera of gram-positive rods, such as *Arthrobacter* and *Brevibacterium*, show also a rod-coccus cycle (1). The genus *Janibacter* is included in the family *Intrasporangiaceae*, order *Actinomycetales*, which belongs to the lineage of the gram-positive bacteria with high guanine-plus-cytosine (G+C) content. This group of microorganisms encompasses aerobically growing, asporogenous, irregularly shaped, non-partially acid-fast, gram-positive rods generally called coryneforms. The term "coryneform" is actually somewhat misleading, since only true *Corynebacterium* spp. exhibit a typical club-shaped morphology, whereas other bacteria such as *Janibacter* spp. show an irregular morphology. Besides *J. limosus*, three species of *Janibacter* have been described. *J. terrae* and *J. brevis* have been isolated from environmentally polluted samples (4, 10). Actually, *J. terrae* and *J. brevis* belong to the same species and *J. brevis* is a later heterotypic synonym of *J. terrae* (6). More recently, *J. melonis* has been isolated from abnormally spoiled oriental melon (11). Thus, all of the previously described species of *Janibacter* have been isolated from the environment. To our knowledge, strain 03-238 is the first to have been isolated from human blood. Preliminary phenotypic tests indicated that this strain, responsible for repeated bacteremia, may belong to the group of coryneform bacteria. In clinical laboratories, the identification of this group of bacteria is usually carried out by

TABLE 1. Phenotypic tests for differentiating between isolate 03-238 of *Janibacter* sp. and related species of *Janibacter*<sup>a</sup>

Test	Result <sup>b</sup> for:			
	Isolate 03-238	<i>J. limosus</i>	<i>J. melonis</i>	<i>J. terrae</i> <sup>c</sup>
Color of colonies	Cream or yellowish	White or yellow	Cream	White, cream, or yellowish
Morphology	Rods or cocci	Rods or cocci	Cocci	Rods or cocci
H <sub>2</sub> S production	–	+	–	V
Nitrate reduction	–	+	+	+
Esculin hydrolysis	–	–	+	–
Gelatin hydrolysis	+	+	–	+

<sup>a</sup> Data are from Imamura et al. (4), Lang et al. (6), Martin et al. (7), and Yoon et al. (10, 11).

<sup>b</sup> +, positive; –, negative; V, variable.

<sup>c</sup> *J. brevis* is a later heterotypic synonym of *J. terrae*.

using identification systems such as the API Coryne strip (bioMérieux) (1, 2). However, in the present case, the identification obtained with API Coryne strip was not satisfactory. Thus, the 16S rRNA gene sequence of the isolate was determined. The comparison of the sequence obtained to all bacterial sequences available from the GenBank database showed that isolate 03-238 belongs to the genus *Janibacter* but is distinct from all of the species of *Janibacter* previously described (Fig. 1). Phenotypic tests, such as H<sub>2</sub>S production, nitrate reduction, esculin hydrolysis, and gelatin hydrolysis, may help to differentiate isolate 03-238 from the previously described *Janibacter* species (Table 1).

This case report shows that *Janibacter* species are not only present in the environment but may also be isolated from clinical specimens. The leukemia and the anticancerous chemotherapy administration were probably major risk factors for the dissemination of isolate 03-238 in blood. Thus, this species of *Janibacter* may be considered an opportunistic pathogen responsible for bacteremia in immunocompromised hosts. We assume that the intravascular catheterization triggered the penetration of the bacteria through the patient's skin and its spread to the bloodstream. As for other coryneform bacteria, *Janibacter* species may be present on human skin. However, the natural habitat of this human strain of *Janibacter* is at present unknown and remains to be determined. Antimicrobial susceptibility of previous isolates of *Janibacter* has not been studied according to current guidelines used in clinical laboratories. MIC results obtained for isolate 03-238 indicate that penicillins may be more active in vitro than cephalosporins. Such a result has been reported previously for *Arthrobacter* spp. (1). Isolate 03-238 was also susceptible to the tested aminosides, fluoroquinolones, and glycopeptides. Thus, the antibiotherapy including piperacillin and tobramycin was an adequate treatment and may have contributed to the control of the infection. In conclusion, the human source, the phenotypic tests, and the 16S rRNA gene sequence of isolate 03-238 suggest that this isolate may belong to a novel species of *Janibacter*. We refrained from naming this probable novel species, since a single

strain is presently available. Strain 03-238 has been deposited in two collections (DSM 15959 and CIP 108123). Moreover, this case highlights the value of PCR amplification, sequencing, and comparison to 16S rRNA gene sequence databases for the identification of uncommon or novel pathogens. Clinical microbiologists must be aware of the opportunistic pathogenicity of this newly described species, which deserves further studies to confirm that it constitutes a novel species of *Janibacter*.

**Nucleotide sequence accession number.** The 16S rRNA gene sequence of isolate 03-238 of *Janibacter* sp. has been deposited in the GenBank database under accession number AY383745.

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