

Corynebacterium freneyi Bacteremia

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***Corynebacterium freneyi* is a recently described alpha-glucosidase-positive species of the genus *Corynebacterium*. To our knowledge, there is no description of human infection due to this species. We report on a case of bacteremia due to *C. freneyi* following vascular surgery.**

CASE REPORT

Among the corynebacteria, *Corynebacterium xerosis*, *Corynebacterium amycolatum*, and *Corynebacterium striatum* are the species that are the most frequently isolated from clinical samples (8). Their natural habitat is human skin, and as a result, these species sometimes appear as sample contaminants. These species are frequently misidentified by biochemical identification (5, 6, 12, 14). *Corynebacterium freneyi* is closely related to these three species and was recently described by Renaud et al. (9). Those investigators have studied five strains isolated from clinical samples, but to our knowledge, there is no description of human infection due to this species. We report on a case of bacteremia due to *C. freneyi* after vascular surgery.

A 49-year-old man was hospitalized in April 2001 and June 2001 for acute ischemia of the right tibial artery and surgical recanalization. In August 2001, he suffered of acute pain in decubitus position. Upon examination, his feet were cold and had decreased sensitivity. On 18 August 2001, he received a graft of the cephalic left vein to create a femoral-pedal bridge. On 20 August, he suffered acute ischemia of his right leg and the leg was amputated at the metatarsal level. On the same day, he presented with a temperature of 38.5°C, and one pair of blood samples for culture (one sample for aerobic culture and one sample for anaerobic culture) were drawn and subcultured on sheep blood agar (bioMérieux, Marcy-l'Etoile, France) at 37°C. After blood culture and susceptibility testing, the patient was started on intravenous amoxicillin at 2 g/day. Apyrexia was obtained within 48 h. The patient was discharged on 27 August to a rest home.

In the case of our patient, after a culturing time of 48 h, 1-mm whitish colonies with irregular edges were observed from both the aerobic and the anaerobic blood culture bottles. Gram staining revealed gram-positive, non-spore-forming diphtheroids. Tests for catalase and alpha-glucosidase were positive. The API-CORYNE (bioMérieux) profile was 3110325, coding for *C. striatum*-*C. amycolatum* (API-CORYNE profiles book, 2nd ed., 1997).

Antibiotic susceptibility testing was performed on sheep

blood Mueller-Hinton agar plates by the disk diffusion method according to the recommendations of the NCCLS (7). The MICs were 1 mg/liter for amoxicillin, 0.5 mg/liter for rifampin, 1 mg/liter for gentamicin, 2 mg/liter for vancomycin, 8 mg/liter for erythromycin, and 16 mg/liter for co-trimoxazole.

Further identification of the isolate was done by 16S rRNA gene sequence analysis. The DNA of a single colony was extracted by using the Fast-prep DNA extraction kit and the Fast-prep DNA device as described by the supplier (Bio 101, Inc., La Jolla, Calif.) (3). The 16S rRNA gene was amplified by using primers FD1 (5'-AGAGTTTGATCCTGGCTGAG-3') and RP2 (5'-ACGGCTACCTTGTACGACTT-3') (15). PCRs were performed with a Perkin-Elmer 9600 thermocycler under the following conditions: following a first denaturation step (95°C for 2 min), a three-step cycle of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min was repeated 35 times. Sequence determination was performed as described previously (2). The partial sequence (1,225 bp) of the 16S rRNA of this isolate was deposited in GenBank (accession number AY210513) and was aligned and compared with all eubacterial 16S rRNA gene sequences available in the GenBank and EMBL databases by multisequence analysis with the advanced BLAST software of the National Center for Biotechnology Information (1). The highest 16 rRNA gene sequence similarity value (99.7%) was obtained with the *C. freneyi* CIP106767^T 16S rRNA gene sequence (EMBL AJ292762). The morphology and the biochemical characteristics of our isolate were similar to those of type strain CIP106767 studied by Renaud et al. (9). Following initial alignment of the sequences with the CUSTAL W program (version 1.8) (13), neighbor-joining analysis was performed by using PAUP software (version 4.0b1; Sinauer, Sunderland, Mass.). Figure 1 shows the dendrogram that we obtained. Our isolate, isolate 116594, is closely related to *C. freneyi* CIP106767^T and *C. freneyi* CIP106768. The sequence of *C. xerosis* is the most similar to that of *C. freneyi* CIP106768 (similarity, 98.5%), followed by those of *C. amycolatum* (similarity, 98.0%), *Corynebacterium asperum* (similarity, 97.0%), *Corynebacterium efficiens* (similarity, 96.1%) (11), *C. striatum* (similarity, 94.0%), *Corynebacterium argentoratense* (similarity, 93.8%) (10), and *Corynebacterium felinum* (similarity, 92.0%) (4). These results are similar to those of Renaud et al. (9).

The isolate grew readily in pure culture, and no other *C. freneyi* strain was isolated in the same laboratory. The isolation of *C. freneyi* from a blood culture has never been reported. The isolate was recovered from blood when the patient presented with an acute onset of fever, with no other microorganism

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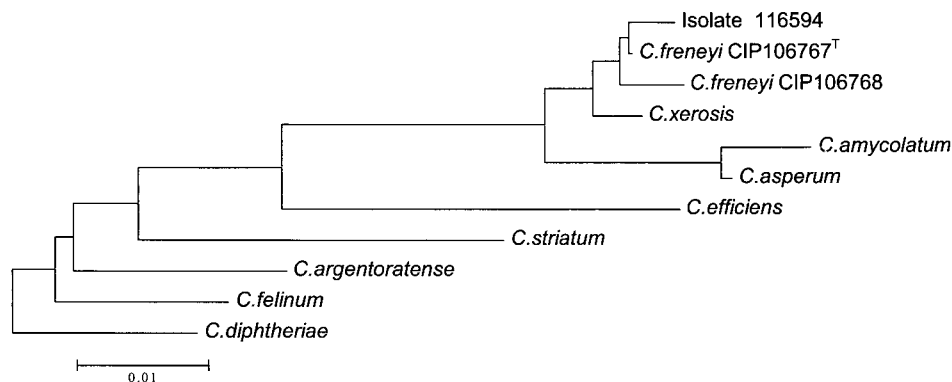


FIG. 1. Dendrogram obtained by analysis of 16S rRNA gene sequences. *Corynebacterium diphtheriae* was used as the outgroup.

recovered from other specimens obtained at appropriate times. These facts suggest that this isolate was not a contaminant. This description could be relevant for infectious disease consulting.

Analysis of 16S rRNA gene sequences offers a reliable and straightforward tool for organism identification (12), and routine use of this method should increase our knowledge regarding the clinical spectrum of *C. freneyi* infections in humans.

Nucleotide sequence accession number. The partial sequence (1,225 bp) of the 16S rRNA of patient isolate 116594 has been deposited in GenBank under accession number AY210513.

REFERENCES

- Altschul, X., F. Stephen, L. Thomas, X. Madden, A. Alejandro, X. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Beau, F., C. Bollet, T. Coton, E. Garnotel, and M. Drancourt. 1999. Molecular identification of a *Nocardiosis dassonvillei* blood isolate. *J. Clin. Microbiol.* **37**:3366–3368.
- Cheung, A. L., K. J. Eberhardt, and V. A. Fischetti. 1994. A method to isolate RNA from gram-positive bacteria and mycobacteria. *Anal. Biochem.* **222**: 511–514.
- Collins, M. D., L. Hoyle, R. A. Hutson, G. Foster, and E. Falsen. 2001. *Corynebacterium testudinoris* sp. nov., from a tortoise, and *Corynebacterium felinum* sp. nov., from a Scottish wild cat. *Int. J. Syst. Evol. Microbiol.* **51**: 1349–1352.
- Funke, G., P. A. Lawson, K. A. Bernard, and M. D. Collins. 1996. Most *Corynebacterium xerosis* strains identified in the routine clinical laboratory correspond to *Corynebacterium amycolatum*. *J. Clin. Microbiol.* **34**:1124–1128.
- Lagrou, K., J. Verhaegen, M. Janssens, G. Wauters, and L. Verbist. 1998. Prospective study of catalase-positive coryneform organisms in clinical specimens: identification, clinical relevance, and antibiotic susceptibility. *Diagn. Microbiol. Infect. Dis.* **30**:7–15.
- National Committee for Clinical Laboratory Standards. 1990. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Renaud, F. N., M. Dutaur, S. Daoud, D. Aubel, P. Riegel, D. Monget, and J. Freney. 1998. Differentiation of *Corynebacterium amycolatum*, *C. minutissimum*, and *C. striatum* by carbon substrate assimilation tests. *J. Clin. Microbiol.* **36**:3698–3702.
- Renaud, F. N., D. Aubel, P. Riegel, H. Meugnier, and C. Bollet. 2001. *Corynebacterium freneyi* sp. nov., alpha-glucosidase-positive strains related to *Corynebacterium xerosis*. *Int. J. Syst. Evol. Microbiol.* **51**:1723–1728.
- Riegel, P., R. Ruimy, D. De Briel, G. Prevost, F. Jehl, F. Bimet, R. Christen, and H. Monteil. 1995. *Corynebacterium argentoratense* sp. nov., from the human throat. *Int. J. Syst. Bacteriol.* **45**:533–537.
- Seto, A., K. Yamada, E. Kimura, T. Nakamatsu, A. Hiraishi, and S. Yamanaka. 2002. *Corynebacterium efficiens* sp. nov., a glutamic-acid-producing species from soil and vegetables. *Int. J. Syst. Evol. Microbiol.* **52**:1127–1131.
- Tang, Y. W., A. Von Graevenitz, M. G. Waddington, M. K. Hopkins, D. H. Smith, H. Li, C. P. Kolbert, S. O. Montgomery, and D. H. Persing. 2000. Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J. Clin. Microbiol.* **38**:1676–1678.
- Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673–4680.
- Wauters, G., B. Van Bosterhaut, M. Janssens, and J. Verhaegen. 1998. Identification of *Corynebacterium amycolatum* and other nonlipophilic fermentative corynebacteria of human origin. *J. Clin. Microbiol.* **36**:1430–1432.
- Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**:697–703.