Case Report

Interstitial pulmonary inflammation due to *Microbacterium* sp. after heart transplantation

Giovanni M. Giammanco,¹ Sarina Pignato,² Patrick A. D. Grimont,³ Francine Grimont,³ Carmelita Santangelo,⁴ Giuseppe Leonardi,⁵ Angelo Giuffrida,⁵ Vivian Legname⁵ and Giuseppe Giammanco²

Correspondence

Giovanni M. Giammanco
g.m.giammanco@unipa.it

¹Dipartimento di Igiene e Microbiologia 'G. D'Alessandro', Università di Palermo, I-90127 Palermo, Italy

²Dipartimento 'G. F. Ingrassia' Igiene e Sanità Pubblica, Università di Catania, I-95100 Catania, Italy

³Unité Biodiversité Bactéries Pathogènes Emergentes, Institut Pasteur, F-75724 Paris, France

^{4,5}Servizio di Patologia Clinica, Sezione di Batteriologia⁴, and Divisione di Cardiocirurgia⁵, Azienda Ospedali Vittorio Emanuele, Ferrarotto, Santo Bambino, I-95100 Catania, Italy

Received 30 June 2005
Accepted 26 October 2005

A coryneform bacterium was isolated from the bronchoalveolar aspirate of a patient with interstitial pulmonary inflammation. Commercial systems identified the isolate as *Corynebacterium* sp. or *Aureobacterium* sp./*Corynebacterium aquaticum*, but 16S rRNA gene analysis unequivocally attributed it to the genus *Microbacterium*. This represents the first documented case of *Microbacterium* pulmonary infection.

Case report

A 44-year-old man underwent heart transplantation on 8 August 2000 as a consequence of ischaemic cardiomyopathy (previous extended anterior myocardial infarction). Transplantation and the post-operative course were uncomplicated and the patient was discharged on 31 August 2000 and prescribed a cyclosporin A, azathioprine and corticosteroid therapy (cyclosporin blood levels were expected to be 400 ng dl⁻¹). Ten days later, the patient showed an acute organ rejection documented by endomyocardial biopsy (Grade 2, Dallas Classification, with diffuse and aggressive infiltrate) and was treated with high doses of intravenous corticosteroid (500 mg methylprednisolone per day for 3 days). Following this therapy, the rejection successfully reversed (Grade 1A at 1 month after treatment).

Twelve days after corticosteroid treatment, the patient was readmitted with fever, neck pain, oesophageal discomfort during deglutition and a cough. At that time, the white blood cell count was 0.56 cells µl⁻¹ with 80 % neutrophils, 15 % lymphocytes and a CD4/CD8 ratio of 0.4, thus showing intense immunosuppression. Fever quickly rose to 38.3 °C. Several blood and sputum cultures were negative for aerobic and anaerobic bacteria and for fungal cells. Before and after the transplant, the patient had been monitored for cytomegalovirus by the pp65 antigenaemia

test, whose results were constantly negative. At the time of readmission, a further test for cytomegalovirus pp65 antigenaemia was performed and the result again was negative. A chest radiograph showed interstitial inflammation of the inferior left pulmonary lobe. After a few days, pleural effusion developed, as evidenced by physical examination. Computerized axial tomography examination confirmed pulmonary inflammation and pleural effusion, but excluded mediastine involvement. Initially, the patient was treated empirically with meropenem (500 mg three times per day), clarithromycin (500 mg per day), ciprofloxacin (200 mg twice a day) and fluconazole (150 mg per day), all intravenously, and oral trimethoprim/sulfamethoxazole (800 mg per day). This therapy did not lower the fever, nor did it attenuate the other symptoms. After 5 days, a selective bronchoalveolar washing was performed and the aspirate was examined. Direct Gram staining showed the presence of Gram-positive bacilli, whilst observation for *Pneumocystis jirovecii* (*Pneumocystis carinii*) after Giemsa and toluidine blue O staining yielded negative results. The aspirate was not checked for the presence of cytomegalovirus, but was cultured for aerobic and anaerobic bacteria, as well as for fungi. Culture on blood agar and MacConkey agar under aerobic conditions yielded growth in pure culture of a Gram-positive coryneform bacterium that was found to be sensitive to vancomycin. Previous antibiotics were replaced by vancomycin (500 mg four times per day) and this therapy was prolonged for 20 days until complete resolution of radiographic and clinical symptoms. The patient was discharged after 31 days in a good condition.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the OVE isolate reported in this paper is AY960683.

Microbiological studies

Colonies of the isolate (OVE isolate) were non-haemolytic on 5% sheep blood agar and showed a bright yellow pigmentation at room temperature. The isolate was lactose-non-fermenting, catalase-positive and oxidase-negative. The Vitek 2 identification system (bioMérieux) identified the strain as *Corynebacterium* sp., whilst the API Coryne system (bioMérieux) identified it as *Aureobacterium* sp./*Corynebacterium aquaticum* with a 98.9% level of confidence. However, in contrast to the reported reactions for *C. aquaticum* (Funke *et al.*, 1994; Grove *et al.*, 1999), the isolate did not reduce nitrate and was non-motile. Using the API Coryne system, the isolate gave positive results for pyrazinamidase, alkaline phosphatase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and aesculin hydrolysis. It gave negative results for nitrate reduction, pyrrolidonyl arylamidase, β -glucuronidase, hydrolysis of urea, and fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycerol. Hydrolysis of gelatin was negative using API Coryne, but positive by inoculation on nutrient gelatin medium (Difco) after 3 days of incubation at 22 °C. The numerical profile of the isolate on the API Coryne system was 2570004, similar to the 2550004 profile obtained by the same identification system with the type strain (CIP 102402^T) of *Aureobacterium liquefaciens* (reclassified as *Microbacterium liquefaciens*) and to the 5570004 profile obtained with the type strain (CIP 64.13^T) of *C. aquaticum* (reclassified as *Leifsonia aquatica*). The main biochemical characteristics of the OVE clinical isolate are shown in Table 1, compared with those reported in the literature for the two above-mentioned species. Manual analysis of the overall reactions produced by the two identification systems used allowed us tentatively to identify our

isolate as *A. liquefaciens*, which is not included in the identification database of the identification systems considered above. According to the Vitek 2 system, Gram-positive Susceptibility Test Card AST-GP61 and Gram-negative Susceptibility Test Cards AST-GN08 and AST-GN11, the isolate was susceptible to cefepime, ceftriaxone, cefuroxime, cephalothin, erythromycin, imipenem, piperacillin, rifampicin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin, but was resistant to ceftazidime, gentamicin, meropenem and oxacillin. The MICs for imipenem and meropenem were also determined by the Etest (AB BIODISK) and were 6 and 16 mg l⁻¹, respectively.

In order to determine the exact phylogenetic position of the OVE isolate, the 16S rRNA gene was amplified by PCR and sequenced by GenomeExpress (Montreuil, France). The 16S rRNA gene sequence of our isolate was used for a BLAST search in the EMBL/GenBank database and showed high similarity with members of the genus *Microbacterium*. The partial 16S rRNA gene sequence (1446 bp) of our isolate was further compared and aligned to published 16S rRNA gene sequences searched with the taxonomy browser of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and retrieved from GenBank. The retrieved 16S rRNA gene sequences belonged to the type strains of 27 different species in the genus *Microbacterium* and to the *L. aquatica* type strain (Fig. 1). The EMBL/GenBank accession numbers for the 16S rRNA sequences are given in Fig. 1.

Sequence alignment was performed using CLUSTAL_W, and phylogenetic analysis, including calculation of percentage sequence similarity, construction of a phylogenetic tree and an assessment of the tree topology by bootstrap analysis, was

Table 1. Phenotypic characteristics of the study isolate and available information for closely related *Microbacterium* spp. and *L. aquatica* (Behrendt *et al.*, 2001; Funke *et al.*, 1994; Grove *et al.*, 1999; Laffineur *et al.*, 2003; Lau *et al.*, 2002; Nolte *et al.*, 1996; Schumann *et al.*, 1999)

+, Positive; -, negative; (+), delayed positive; v, variable; NA, not available; y, yellow; yw, yellow/white; GEL, gelatin; URE, urea; AES, aesculin; GLU, glucose; RIB, ribose; XYL, xylose; MAN, mannitol; MAL, maltose; LAC, lactose; SAC, sucrose; GLY, glycerol.

	Colour of colony	Motility	Hydrolysis of:			Nitrate reduction	Acid from:							
			GEL	URE	AES		GLU	RIB	XYL	MAN	MAL	LAC	SAC	GLY
OVE isolate*	y	-	+†	-	+	-	-	-	-	-	-	-	-	-
<i>A./M. liquefaciens</i>	y	-	+	-	+	v	-	NA	NA	NA	NA	NA	NA	NA
<i>C. aquaticum/L. aquatica</i>	y	+	-	-	+	+	+	NA	+	+	v	+	v	NA
<i>M. foliorum</i>	y	+	+	-	+	-	+	+	+	+	+	-	+	-
<i>M. keratanolyticum</i>	y	+	+	-	+	+	-	NA	NA	NA	NA	NA	NA	NA
<i>M. luteolum</i>	yw	-	-	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA
<i>M. oxydans</i>	y	+	+	-	+	-	+	NA	v	+	+	v	+	+
<i>M. paraoxydans</i>	y	+	+	NA	(+)	NA	+	NA	NA	+	+	NA	+	NA
<i>M. phyllosphaerae</i>	y	+	+	-	+	-	v	+	+	+	+	-	+	-
<i>M. saperdae</i>	yw/y	+	-	-	+	-	+	NA	-	+	+	NA	+	NA

*Characters determined by the Vitek and API Coryne systems, with the exception of colour of colony and motility.

†Gelatin hydrolysis was negative in the API Coryne system and was observed only by inoculation on nutrient gelatin medium.

Fig. 1. Neighbour-joining phylogenetic dendrogram based on comparison of the 16S rRNA gene sequences of the OVE clinical isolate and type strains of *Microbacterium* species and *L. aquatica*. Reference sequences were obtained from the GenBank/EMBL databases; accession numbers are given in parentheses. Bootstrap values are given at branch nodes. The bar indicates the percentage of nucleotide substitutions.

carried out using MEGA software version 3.0 (Kumar *et al.*, 2004), using the Kimura two-parameter model as a method of substitution and the neighbour-joining method to construct the phylogenetic tree. In the phylogenetic tree (Fig. 1), the OVE clinical isolate clustered together with *Microbacterium oxydans* (98.9% sequence similarity), *Microbacterium luteolum* (98.8%), *Microbacterium foliorum* (98.8%), *Microbacterium phyllosphaerae* (98.7%), *M. liquefaciens* (98.6%), *Microbacterium saperdae* (98.6%), *Microbacterium paraoxydans* (98.6%) and *Microbacterium keratanolyticum* (98.1%), whilst it showed only 93.8% similarity to the *L. aquatica* sequence. Although low bootstrap values were obtained with 1000 pseudoreplicate datasets, the clustering was confirmed by the unweighted pair group method with arithmetic averages and the minimum evolution method (data not shown).

Discussion

In recent years, a limited number of infections caused by Gram-positive coryneform bacteria identified as *C. aquaticum*, *Aureobacterium* spp. (particularly *A. liquefaciens*),

Microbacterium spp. or CDC coryneform group A-4 and A-5 have been described (Funke *et al.*, 1997a; Grove *et al.*, 1999; Lau *et al.*, 2002). In particular, only three detailed clinical descriptions of *Aureobacterium* spp. infections have been published, whilst a larger number of clinical isolates of this micro-organism have been described (Funke *et al.*, 1994, 1997b, 1998; Grove *et al.*, 1999; Nolte *et al.*, 1996; Saweljew *et al.*, 1996). The first report of the isolation of *Aureobacterium* spp. from clinical specimens was published by Funke *et al.* (1994), who were able to identify seven strains of this unusual pathogen among 11 clinical isolates of yellow-pigmented Gram-positive coryneform bacteria, tentatively identified as *C. aquaticum*. The isolates were obtained from a variety of clinical samples: drainage of a polytraumatized patient, blood culture, cerebrospinal fluid, peritoneal fluid from a patient submitted to chronic ambulatory dialysis, abdominal deep wound of a kidney/pancreas-transplanted patient, epidural abscess and a soft-tissue infection. Unfortunately, no other clinical details were reported. At present, only three reports have been published in which clinical details of *Aureobacterium* infections are described (Grove *et al.*, 1999; Nolte *et al.*, 1996; Saweljew *et al.*, 1996). In

addition, isolates belonging to the genus *Microbacterium* have rarely been encountered in clinical specimens; of six cases reviewed by Funke *et al.* (1997a), three were endophthalmitis, two were septicaemic infections and one was mitral valve endocarditis. A nosocomial outbreak of *Microbacterium* bacteraemia involving six oncological patients has been described by Alonso-Echanove *et al.* (2001). The first documented case of catheter-related *Microbacterium* bacteraemia was reported by Lau *et al.* (2002). Formal identification at the species level and description of *M. paraoxydans* was achieved by Laffineur *et al.* (2003) for a yellow-pigmented coryneform rod isolated from the blood of a patient with leukaemia. In almost all described *Aureobacterium* or *Microbacterium* infections, patients were immunocompromised and/or suffering debilitating diseases. In our case, the patient was also immunodeficient because of corticosteroid treatment following heart transplantation. A Gram-positive coryneform bacterium was isolated from his bronchoalveolar aspirate in pure culture, suggesting that the isolate was responsible for the interstitial pulmonary inflammation. The patient recovered after treatment with vancomycin, which had been selected according to the results of *in vitro* susceptibility testing of the isolate. The case of *Aureobacterium* infection described by Grove *et al.* (1999) was treated empirically with ceftazidime, gentamicin and imipenem, but the patient recovered only after vancomycin was added.

The isolate from our patient was identified as *Corynebacterium* sp. and *Aureobacterium* sp./*C. aquaticum* by two different commercial identification systems. At present, *Aureobacterium* species have been proposed for inclusion in the genus *Microbacterium* (Takeuchi & Hatano, 1998), whilst *C. aquaticum* is classified in the genus *Leifsonia* (Evtushenko *et al.*, 2000). *C. aquaticum* has been defined as strictly motile (Funke *et al.*, 1997b; Leifson, 1962), whilst our isolate was non-motile. Furthermore, the numerical profile obtained by the API Coryne system was very similar to that shown by the type strain of *A. liquefaciens* (reclassified as *M. liquefaciens*), whereas it clearly differed from that of the type strain of *C. aquaticum* (reclassified as *L. aquatica*). Finally, our clinical isolate proved genotypically distant from the registered sequence of this latter species. In contrast, BLAST and phylogenetic analyses showed high 16S rRNA gene similarities with some species of the genus *Microbacterium*, namely *M. oxydans*, *M. luteolum*, *M. foliorum*, *M. phyllosphaerae*, *M. liquefaciens* (formerly *A. liquefaciens*), *M. saperdae*, *M. paraoxydans* and *M. keratanolyticum*, the closest phylogenetic neighbours with 98.9–98.1 % similarity. On the basis of these results, the isolate can be classified in the genus *Microbacterium*. With the exception of *M. liquefaciens*, at least two phenotypic characteristics differentiated our isolate from the most closely related *Microbacterium* species (Table 1 and references therein), but, unfortunately, the biochemical data available from the literature are incomplete. Since genetic distances among species seem to be very low in the genus *Microbacterium* (Funke *et al.*, 1998;

Schumann *et al.*, 1999), it is possible that our isolate represents a novel species.

Although Laffineur *et al.* (2003) reported a *Microbacterium* isolate from a bronchial aspirate, to our knowledge, this is the first detailed study regarding the isolation of a *Microbacterium* sp. from interstitial pulmonary inflammation. The isolation in pure culture in the absence of other pathogens suggests that the isolate was responsible for the inflammation. Laboratories should always be alert to the possibility of a novel organism when different commercial or conventional systems do not agree on the identification of an isolate. Our results confirm that 16S rRNA gene sequencing provides a powerful tool for the definitive identification of clinical coryneform bacterial isolates (Funke *et al.*, 1994; Grove *et al.*, 1999; Laffineur *et al.*, 2003; Lau *et al.*, 2002; Tang *et al.*, 2000).

References

- Alonso-Echanove, J., Shah, S. S., Valenti, A. J., Dirrigli, S. N., Carson, L. A., Arduino, M. J. & Jarvis, W. R. (2001). Nosocomial outbreak of *Microbacterium* species bacteremia among cancer patients. *J Infect Dis* **184**, 754–760.
- Behrendt, U., Ulrich, A. & Schumann, P. (2001). Description of *Microbacterium foliorum* sp. nov. and *Microbacterium phyllosphaerae* sp. nov., isolated from the phyllosphere of grasses and the surface litter after mulching the sward, and reclassification of *Aureobacterium resistens* (Funke *et al.* 1998) as *Microbacterium resistens* comb. nov. *Int J Syst Evol Microbiol* **51**, 1267–1276.
- Evtushenko, L. I., Dorofeeva, L. V., Subbotin, S. A., Cole, J. R. & Tiedje, J. M. (2000). *Leifsonia poae* gen. nov., sp. nov., isolated from nematode galls on *Poa annua*, and reclassification of '*Corynebacterium aquaticum*' Leifson 1962 as *Leifsonia aquatica* (ex Leifson 1962) gen. nov., nom. rev., comb. nov. and *Clavibacter xyli* Davis *et al.* 1984 with two subspecies as *Leifsonia xyli* (Davis *et al.* 1984) gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 371–380.
- Funke, G., von Graevenitz, A. & Weiss, N. (1994). Primary identification of *Aureobacterium* spp. isolated from clinical specimens as '*Corynebacterium aquaticum*'. *J Clin Microbiol* **32**, 2686–2691.
- Funke, G., Haase, G., Schnitzler, N., Schrage, N. & Reinert, R. R. (1997a). Endophthalmitis due to *Microbacterium* species: case report and review of microbacterium infections. *Clin Infect Dis* **24**, 713–716.
- Funke, G., von Graevenitz, A., Clarridge, J. E., 3rd & Bernard, K. A. (1997b). Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* **10**, 125–159.
- Funke, G., Lawson, P. A., Nolte, F. S., Weiss, N. & Collins, M. D. (1998). *Aureobacterium resistens* sp. nov., exhibiting vancomycin resistance and teicoplanin susceptibility. *FEMS Microbiol Lett* **158**, 89–93.
- Grove, D. I., Der-Haroutian, V. & Ratcliff, R. M. (1999). *Aureobacterium* masquerading as '*Corynebacterium aquaticum*' infection: case report and review of the literature. *J Med Microbiol* **48**, 965–970.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Laffineur, K., Avesani, V., Cornu, G., Charlier, J., Janssens, M., Wauters, G. & Delmée, M. (2003). Bacteremia due to a novel *Microbacterium* species in a patient with leukemia and description of *Microbacterium paraoxydans* sp. nov. *J Clin Microbiol* **41**, 2242–2246.

- Lau, S. K. P., Woo, P. C. Y., Woo, G. K. S. & Yuen, K.-Y. (2002).** Catheter-related *Microbacterium* bacteremia identified by 16S rRNA gene sequencing. *J Clin Microbiol* **40**, 2681–2685.
- Leifson, E. (1962).** The bacterial flora of distilled and stored water. III. New species of the genera *Corynebacterium*, *Flavobacterium*, *Spirillum*, and *Pseudomonas*. *Int Bull Bacteriol Nomencl Taxon* **12**, 161–170.
- Nolte, F. S., Arnold, K. E., Sweat, H., Winton, E. F. & Funke, G. (1996).** Vancomycin-resistant *Aureobacterium* species cellulitis and bacteremia in a patient with acute myelogenous leukemia. *J Clin Microbiol* **34**, 1992–1994.
- Saweljew, P., Kunkel, J., Feddersen, A., Baumert, M., Baehr, J., Ludwig, W., Bhakdi, S. & Husmann, M. (1996).** Case of fatal systemic infection with an *Aureobacterium* sp.: identification of isolate by 16S rRNA gene analysis. *J Clin Microbiol* **34**, 1540–1541.
- Schumann, P., Rainey, F. A., Burghardt, J., Stackebrandt, E. & Weiss, N. (1999).** Reclassification of *Brevibacterium oxydans* (Chatelain and Second 1966) as *Microbacterium oxydans* comb. nov. *Int J Syst Bacteriol* **49**, 175–177.
- Takeuchi, M. & Hatano, K. (1998).** Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* **48**, 739–747.
- Tang, Y.-W., Von Graevenitz, A., Waddington, M. G., Hopkins, M. K., Smith, D. H., Li, H., Kolbert, C. P., Montgomery, S. O. & Persing, D. H. (2000).** Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J Clin Microbiol* **38**, 1676–1678.