**Case Report**

**Difficulty with *Gordonia bronchialis* identification by Microflex mass spectrometer in a pacemaker-induced endocarditis**

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**Introduction:** This report describes the first case, to the best of our knowledge, of pacemaker-induced endocarditis due to *Gordonia bronchialis*.

**Presentation:** Pacemaker-induced endocarditis due to *G. bronchialis* infection was determined in a 92-year old man. This Gram-positive bacillus failed to be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry technology, whereas the taxon was indexed in the database. 16S rRNA and rpoB gene sequencing were required to determine the correct strain identity.

**Conclusion:** Infections caused by *G. bronchialis* remain a rare phenomenon affecting immunocompromised patients and/or medical device carriers. Molecular tools may be necessary to ensure accurate identification.

**Keywords:** 16S rRNA; endocarditis; *Gordonia bronchialis*; MALDI-TOF MS; pacemaker; rpoB sequencing.

**Case report**

A 92-year old man who underwent pacemaker implantation 20 years ago was referred to our institution for management of a local pocket infection. The patient’s medical history included high blood pressure, atrial fibrillation and pulmonary embolism. A first management consisting of local surgical intervention and antimicrobial therapy (pristinamycin-oxacillin) had been performed the previous month in another hospital. On admission, physical examination revealed blood pressure at 120/75 mmHg with cardiac frequency at 78 beats min-1 and a grade III New York Heart Association dyspnoea. The patient had no peripheral stigmata of endocarditis. The leukocyte count was $6 \times 10^3 \, \text{mm}^3$ and his C-reactive protein level was 3 mg l-1 (normal value $<10 \, \text{mg} \, \text{l}^{-1}$). As no fever was noted, no blood culture was performed. Transthoracic echocardiography showed a vegetation (12 × 5 mm) on the ventricular lead in the right auricle. As the local infective process did not heal, and according to the echocardiography data, it was decided to rapidly remove the whole pacemaker (generator and leads) without further bacteriological investigations (blood culture). Different parts of the device were plated onto chocolate and 5% sheep blood agar for aerobic culture and inoculated into broths for anaerobic culture as described previously (Klug et al., 2004). After 2 days of incubation, aerobic cultures from all samples grew on blood agar with small dry greyish colonies with a wrinkled aspect in pure culture. The colonies were non-haemolytic, did not produce any aerial hyphae and showed a yellow orange pigmentation after 4 days of incubation. This beaded Gram-positive bacillus was catalase positive and oxidase negative.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification was performed on a 96-target plate (Bruker Daltonics) with a Microflex mass spectrometer (Bruker Daltonics) using FlexControl software (version 3.0). The spectrum was imported and compared with the database by the BioTyper software (version 2.0; Bruker). The BioTyper database contains spectra of approximately 4613 species and is regularly updated by the manufacturer. Theoretically, an accurate identification score to species level is given by a score value $>2.0$ according Bruker recommended cut-off values. The first result was not conclusive with a score of $<1.5$. The strain was retested after a protein extraction step, as described previously (Bizzini et al., 2010; Khot et al., 2012). *Arthrobacter castellii* (score=1.967) was considered as a reliable identification to species level described previously (Klug et al., 2004). After 2 days of incubation, aerobic cultures from all samples grew on blood agar with small dry greyish colonies with a wrinkled aspect in pure culture. The colonies were non-haemolytic, did not produce any aerial hyphae and showed a yellow orange pigmentation after 4 days of incubation. This beaded Gram-positive bacillus was catalase positive and oxidase negative.

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**Abbreviations:** MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

The GenBank/EMBL/DDBJ accession numbers for the sequences of the *Gordonia bronchialis* strain isolated in this report are KF378766 (16S rRNA gene) and KF378767 (rpoB).
### Table 1. Reported infections attributable to *G. bronchialis*

| Reference          | Type of infection                  | No. cases/sex (F/M)/age (years) | Underlying condition                                      | Fever $\geq 38$ °C | Clinical device | Successive treatments                                                                 | Outcome |
|--------------------|-----------------------------------|----------------------------------|------------------------------------------------------------|---------------------|-----------------|----------------------------------------------------------------------------------------|---------|
| Sng *et al.* (2004) | Bacteraemia and sequestrated lung  | 1F/58                            | Mellitus, asthma                                           | Yes                 | No              | Surgical drainage, 3 months vancomycin+ceftriaxone i.v., 6 weeks AMC p.o.               | Cure    |
| Werno *et al.* (2005) | Recurrent breast abscess           | 1F/43                            | Pituitary adenoma                                          | No data             | No              | 3 Days penicillin+flucloxacillin i.v., 3 days AMC+metronidazole p.o., 12 days doxycycline+dldamycin p.o. Surgical drainage, and 5 months doxycycline p.o. | Recurrence |
| Johnson *et al.* (2011) | Bacteraemia and pleural infection | 1F/52                            | Hodgkin lymphoma, splenectomy, breast cancer               | No                  | Pleural catheter | 4 Days vancomycin+ceftazidime i.v., TMP/SMX+imipenem i.v., TMP/SMX p.o. switched by ciprofloxacin+minocycline p.o. for 3 months | Cure    |
| Siddiqui *et al.* (2012) | Osteomyelitis                     | 1F/22                            | Knee surgery                                               | No                  | Bioresorbable screw | Screw removal, 2 weeks vancomycin i.v., 4 weeks ciprofloxacin p.o. | Cure    |
| Wright *et al.* (2012) | Sternal wound infection           | 3M/56–80                         | Mellitus for 2 patients                                    | No                  | Coronary-artery bypass (grafts) | 41–77 Days (mean 60) imipenem i.v. (for the 3 patients) debridement 8 weeks moxifloxacin+linezolid+minocycline p.o. (for 1 patient) | Cure    |
| This case          | Pacemaker-induced endocarditis    | 1M/92                            | High blood pressure, Pulmonary embolism                   | No                  | Pacemaker       | Pacemaker removal, 6 days PTZ+daptomycin i.v., 6 weeks amoxicillin i.v.               | Cure    |

F, female; M, male; AMC, amoxicillin-clavulanic acid; TMP/SMX, trimethoprim-sulfamethoxazole; PTZ, piperacillin-tazobactam; p.o., per os; i.v., intravenous.
according to the recently score proposed by Alatoom et al. (2012). However, the discrepancy between colonial aspects (i.e. smooth blade yellow colonies for Arthrobacter (Heyman et al., 2005) and wrinkled yellow orange colonies for our isolate) led us to perform 16S rRNA gene bacterial sequencing using the primers described by Gauduchon et al. (2003). The 471 bp fragment obtained and compared with GenBank sequences using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) showed 99 % identity with Gordonia bronchialis strain X0217 (GenBank accession no. HQ316182) and Gordonia terrae strain KNUC2111 (GenBank accession no. JN382216).

These results were confirmed by bioinformatic bacterial identification analysis (http://pbil.univ-lyon1.fr/bibi/query.php) with 19 choices for G. bronchialis and one for G. terrae. Sequencing of the rpoB gene was then performed with primers C2700F and C3130R described by Khamis et al. (2004). The 413 bp fragment showed 99 % nucleotide similarity to G. bronchialis strain DSM 43247 (GenBank accession no. CP001802). Antimicrobial susceptibility testing performed by the disc diffusion method on Mueller–Hinton agar with the addition of 5 % blood and interpreted according to EUCAST (2014) criteria showed susceptibility to amoxicillin, piperacillin, cefotaxime, imipenem, aminoglycosides, trimethoprim-sulfamethoxazole, fluoroquinolones, vancomycin and linezolid and resistance to macrolides and tigecycline. According to these data, the first line of antimicrobial treatment by intravenous piperacillin-tazobactam and daptomycin was switched for amoxicillin per os. The clinical outcome was favourable after 6 weeks of treatment.

Discussion

The genus Gordonia, initially named ‘Gordona’ (Tsukamura, 1971), belongs to the suborder Corynebacteriaceae. This taxon became a well-defined genus within the order Actinomycetales (Stackebrandt et al., 1997). Gordonia spp. are aerobic bacteria widely distributed in the environment (soil and water) (Tsukamura, 1971). Based on 10 years of Medline research using Gordonia and endocarditis, this is the first reported case, to the best of our knowledge, of endocarditis due to G. bronchialis in a pacemaker holder. Two other cases of native valve endocarditis were reported due to two other species: Gordonia polysporivorans and Gordonia sp. most closely resembling Gordonia sputi, in two immunocompromised patients (Lesen et al., 2000; Verma et al., 2006). G. bronchialis was clearly involved in seven relevant documented adult infections, described in Table 1 (Sng et al., 2004; Werno et al., 2005; Johnson et al., 2011; Siddiqui et al., 2012; Wright et al., 2012). This bacterium is an opportunistic pathogen inducing a weak immune response from the host. The genus Gordonia is able to form biofilm by producing gordonan, an acidic cell aggregation-inducing polysaccharide, and consequently is responsible for persistent infections (Kondo et al., 2000). In our observation, G. bronchialis was involved in a pacemaker infection in a patient with severe underlying disease. No recommendation for antimicrobial susceptibility testing has been approved. This microorganism appears to be highly susceptible to most antibiotics, as shown by the different regimens applied successfully in the different case reports (Table 1). However, in G. bronchialis infections, prolonged antimicrobial treatment is required, and remission may be attested after a prolonged follow-up period.

More generally, this case underlines the inability of the available identification systems to accurately identify Gordonia spp. This difficulty has largely been commented on in previous articles using the commercial API Coryne system (bioMérieux), which were unable to identify members of the genus Gordonia as it was not indexed in the system’s library (Sng et al., 2004; Werno et al., 2005; Verma et al., 2006). Even with protein extraction, MALDI-TOF MS failed to give the right identification. This might be due to the weak representation of this species in the Bruker library (one spectrum), explaining the misidentification of our isolate as A. castelli (Seng et al., 2009). This technology emerged in recent years as a fast and reliable technique aimed at replacing conventional phenotypic identification methods (Bizzini et al., 2010; Khot et al., 2012). However, the present case leads us to remain critical towards MS results for uncommon Gram-positive bacillus identification, even with good identification scores. Once again (Sng et al., 2004; Werno et al., 2005; Johnson et al., 2011; Siddiqui et al., 2012; Wright et al., 2012), molecular sequencing was required to determine the strain identity. Although 16S rRNA gene sequencing is widely used, the rpoB gene has been described as a reliable molecular marker for Corynebacterium spp. identification (Khamis et al., 2004). In this case, both targets gave the same result.

In conclusion, infections caused by G. bronchialis remain a rare phenomenon affecting immunocompromised patients and/or medical device carriers. Molecular tools may be necessary to ensure accurate identification of this Gram-positive bacterium until there is a wider representation of this species in the Bruker library.

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