Case Report

Pacemaker lead infection and related bacteraemia caused by normal and small colony variant phenotypes of *Bacillus licheniformis*

Evgeny A. Idelevich,1 Christian A. Pogoda,2 Britta Ballhausen,1 Jörg Wüllenweber,1 Lars Eckardt,3 Helmut Baumgartner,4 Johannes Waltenberger,2 Georg Peters1 and Karsten Becker1

Correspondence
Karsten Becker
kbecker@uni-muenster.de

1Institute of Medical Microbiology, University Hospital Münster, Domagkstr. 10, 48149 Münster, Germany
2Department of Cardiology and Angiology, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany
3Division of Electrophysiology, Department of Cardiology and Angiology, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany
4Adult Congenital and Valvular Heart Disease Center, Department of Cardiology and Angiology, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany

Here, we report what we believe to be the first case of bacteraemia with small colony variants of *Bacillus licheniformis* related to a pacemaker lead infection by *B. licheniformis* displaying the normal phenotype. Arbitrarily primed PCR analysis showed a clonal strain. The infection was cured after the removal of the infected device.

Case report

A 42-year-old immunocompetent man was admitted to the Department of Cardiology of the University Hospital Münster for the treatment of pacemaker lead (PL) infection. Fourteen years previously, the patient had undergone mitral valve replacement with an artificial mechanical valve (TEKNA, size 27 mm) after the removal of a papillary fibroelastoma. Due to a post-operative third degree atrioventricular block, a dual-chamber pacemaker (MDT Kappa 401 DR, Medtronic) had been implanted on the right side of the chest. Eight years later, at the age of 36, a left-sided reimplantation of a dual-chamber pacemaker (Biotronik Axios DR, Biotronik) with right atrial (Medtronic CapSureFix 5076, Medtronic) and right ventricular PLs (Biotronik Merox 60-15 BP) became necessary because of PL dysfunction. The old right-sided pacemaker and PL were removed. Three years following reimplantation (i.e. 3 years prior to the present hospitalization), the patient was admitted with sepsis, and *Bacillus licheniformis* was identified in five blood cultures. This was preceded by recurrent pneumonia over 6 months and a 12 kg weight loss. A transoesophageal echocardiogram (TOE) showed no evidence of cardiac infection at that time, and positron emission tomography/computed tomography revealed multiple pulmonary densities. Following antibiotic therapy with amoxicillin/clavulanic acid and gentamicin, laboratory parameters normalized and the patient clinically recovered. Three months prior to the present admission, a partial right-lung lobectomy was performed in another hospital after the radiological detection of lung lesions, and the histopathology showed multiple segmental pulmonary embolism. At that time, several TOEs remained non-diagnostic with regard to infective endocarditis (IE).

Upon the present admission, the patient reported decreased functional capacity during the last 3 years. He suffered from frequent night sweats, orthostatic dizziness and recurrent pulmonary infections. The patient had a reduced general condition. Upon admission, his C-reactive protein level was 52 mg l⁻¹ and the level of leukocytes was 6840 m⁻¹. A TOE revealed a vegetation near the right ventricular PL. Before the initiation of antimicrobial treatment, peripheral blood cultures were collected and three sets grew *B. licheniformis*. Vancomycin and ceftriaxone therapy was initiated. After identification and antimicrobial susceptibility testing, antibiotic therapy was changed to intravenous amoxicillin/clavulanic acid. The pacemaker and the PLs were removed, and *B. licheniformis* was also isolated from both atrial and ventricular PLs. At the same time, a new pacemaker (Medtronic Relia SR RESR01) was placed in an abdominal position using an epicardial PL. A surgical revision was later necessary twice for local haematoma. The four blood cultures taken on

Abbreviations: IE, infective endocarditis; NP, normal phenotype; PL, pacemaker lead; SCV, small colony variant; TOE, transoesophageal echocardiogram.
different days in the post-operative period remained negative. The patient was discharged and continued oral amoxicillin/clavulanic acid for 2 weeks. At 4- and 8-month follow-up visits, the patient showed clinical recovery and a good exercise capacity. C-reactive protein was below the limit of detection and blood cultures remained negative.

The blood cultures obtained from the patient were incubated in the BACTEC 9240 automated blood culture analyser (Becton Dickinson) and positive cultures were plated onto Columbia blood agar, chocolate agar and Schaedler agar with 5% sheep blood. Three sets (both aerobic and anaerobic bottles) became positive and showed Gram-positive rods by microscopy and growth on all three agar plates. Colonies on blood agar appeared small (<1 mm), white and round (Fig. 1). VITEK-2 (bioMérieux) biochemically identified the micro-organism recovered from the blood as *B. licheniformis* and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS with microflex LT system (Bruker and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS with microflex LT system (Bruker Daltonics) yielded the same result. One blood culture positive for *B. licheniformis* was a mixed culture, in which tiny α-haemolytic colonies also grew on blood agar plates, producing green coloration. This organism was identified by the same identification methods as *Granulicatella adiacens*.

Atrial and ventricular leads grew large, irregular, wrinkled, opaque, pleomorphic, ‘licheniform’ colonies on blood agar (Fig. 1). These isolates were also identified unambiguously by both the VITEK-2 and MALDI-TOF systems as *B. licheniformis*. The identification of all isolates was confirmed additionally by 16S rRNA gene sequence analysis, as previously described (Becker et al., 2004; Breitkopf et al., 2005).

Although the isolates from blood and PLs belong to the same species, the morphology and particularly the size of colonies differed considerably (Fig. 1). Pleomorphism of colonies well known for *B. licheniformis* (Logan et al., 2011) was noticeable with each isolate, despite multiple attempts to subcultivate a single colony type. However, the difference in appearance between isolates from blood cultures and those from PLs was tremendous. While the isolates from PLs yielded large colonies typical for *B. licheniformis*, colonies of blood isolates were at least tenfold smaller, resembling the small colony variant (SCV) phenotype as reported for staphylococci and other bacterial pathogens (Proctor et al., 2006). This phenotype remained stable for up to ten passages on solid medium. If grown for 3 days, colonies from blood cultures became only slightly larger (Fig. 1). Also, the phenotype of PL isolates did not change after ten cultural passages. The blood culture isolate grew much slower than the PL isolate and the *B. licheniformis* type strain DSM 13 in the brain heart infusion (BHI) broth at 37 °C and did not achieve the optical density of these two strains, which is indicative for the SCV nature of blood culture isolates, whereas the growth of the PL isolate was, by growth curve analysis, very similar to that of the type strain DSM 13 (data not shown). The biofilm formation was affected in blood culture isolates, as determined in an assay with standing incubation in BHI broth and lysogeny broth for 72 h at 37 °C. Compared with the formation of a biofilm (pellicle) on the broth surface (air–liquid interface) by the PL isolate and the type strain DSM 13, and described previously also for *Bacillus subtilis* by Branda et al. (2006), a pellicle was formed at the bottom of the tube (glass–liquid interface) by the blood culture isolate (data not shown). We did not observe any difference between the isolates in their cell morphology evaluated by the Gram stain and did not see any lack of motility in blood culture isolates. Since the colony morphology and growth characteristics of blood culture isolates were unusual for *B. licheniformis* and differed from the characteristics of PL isolates, we performed genotyping for all *B. licheniformis* isolates of this patient and compared them with the *B. licheniformis* type strain DSM 13, applying arbitrarily primed PCR (AP-PCR), as previously described (Becker et al., 2002; Ellinghaus et al., 1999). AP-PCR has previously been successfully used for heterogeneity determination of *Bacillus* spp. isolates (Hsueh et al., 1999). All isolates had similar patterns, providing evidence that they are clonally identical (Fig. 2). Thus, isolates from blood cultures represent morphologically an SCV phenotype of *B. licheniformis*, with the corresponding parental isolates recovered from PLs displaying the normal phenotype (NP).

Antimicrobial susceptibility testing (AST) was performed using the disc diffusion method and was interpreted according to CLSI breakpoints for *Staphylococcus aureus* (CLSI, 2011), because there are no disc diffusion breakpoints available for *Bacillus* spp. Susceptibility to vancomycin was tested by a gradient MIC method (Etest; bioMérieux) according to the manufacturer’s instructions. AST was
performed with a standard 0.5 McFarland inoculum on Mueller–Hinton agar without supplementation. The growth of blood culture isolates (SCVs) was slightly reduced compared with that of PL isolates (NP isolates). Nevertheless, the growth of both SCV and NP isolates was confluent and readings were possible for both isolate types. When blood culture isolates were streaked onto Mueller–Hinton agar to observe single colony morphology, the colonies were also smaller in comparison with those of PL isolates and the type strain B. licheniformis DSM 13. Thus, the differences between blood culture and PL isolates could be confirmed on this medium. All B. licheniformis isolates were susceptible to amoxicillin/clavulanic acid, cefoxitin, cefazolin, imipenem, gentamicin, amikacin, levofloxacin, erythromycin, vancomycin, rifampicin and trimethoprim–sulfamethoxazole, and were resistant to clindamycin. Interestingly, B. licheniformis SCV blood isolates were susceptible to cefuroxime and cefotaxime, while B. licheniformis NP PL isolates were categorized as intermediate susceptible. Susceptibility testing results of penicillin G and ampicillin were highly variable, most indicated resistance, but some results indicated susceptibility for some SCV and NP isolates. Therefore, the Etest was performed with these agents and the same variability was also noted applying this method. This variability might be related to the extensive growth variability of B. licheniformis.

**Discussion**

*B. licheniformis* is a Gram-positive, rod-shaped, aerobic, endospore-forming organism, which forms colonies of a moderate (2–4 mm) diameter, exceptionally variable in their appearance and often appearing to be mixed cultures (Logan et al., 2011). *B. licheniformis* like other *Bacillus* non-anthracis species is a common contaminant in cultures of clinical material, but numerous reports have described serious disease including ophthalmitis, brain abscess, central venous catheter infections, prosthetic-valve endocarditis and neonatal sepsis (Blue et al., 1995; Jones et al., 1992; Lépine et al., 2009; Santini et al., 1995; Thurn & Goodman, 1988). Quan et al. described a case of pacemaker wire infection caused by *B. licheniformis* (Quan et al., 2000), but, to our best knowledge, SCVs of this pathogen have not been previously reported.

SCVs reported for several bacterial species represent a slow-growing, pathogenic phenotype which facilitates persistent and relapsing infections, such as chronic osteomyelitis, abscesses and foreign body-associated infections (Proctor et al., 2006). Due to their atypical colony morphology and unusual metabolic and proteomic characteristics, SCVs represent a challenge in terms of recovery from clinical specimens, their identification and susceptibility testing (Becker et al., 2006; Kriegeskorte et al., 2011; von Eiff et al., 2006). Best characterized for staphylococcal SCVs, common traits of SCVs are their ability to escape the host’s immune response by intracellular persistence, reduced susceptibilities to antibiotics and enhanced biofilm formation (Baumert et al., 2002; Singh et al., 2010; Tuchscherr et al., 2010).

In the past, a few cases of pacemaker infections associated with staphylococcal SCVs have been described (Maduka-Ezeh et al., 2012; Nielsen et al., 2009; Seifert et al., 2003, 2005; von Eiff et al., 1999), while pacemaker infections caused by SCVs of other species have not been described so far. However, some non-staphylococcal SCVs, such as SCVs of *Brucella melitensis*, *Burkholderia spp.*, *Escherichia coli*, *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*, have been documented as the cause of infections (Proctor et al., 2006). To our best knowledge, this is a first report of a *B. licheniformis* SCV. For other *Bacillus* species, in vitro generation of SCVs has been documented (Maughan & Nicholson, 2011; Rettger & Gillespie, 1933); however, isolation of bacillary SCVs from clinical samples has not been hitherto reported.

Although we isolated *G. adiacens* only in one of three positive blood cultures, it is difficult to distinguish between secondary contamination and mixed infection in this case, and the latter cannot be excluded. Indeed, *G. adiacens*, formerly known as ‘nutritionally variant streptococci’ or ‘satellite streptococci’, is able to cause cardiac infections (Baddour et al., 2005). We also observed its satellite growth on the plate around the colonies of *B. licheniformis* (although no apparent haemolysis was seen around *B. licheniformis* colonies) similar to the well-known satellite growth of *Haemophilus influenzae* in the haemolysis zone of *S. aureus* colonies. It is conceivable that a similar pathogen association may exist in vivo. While the clinical relevance of this additional finding remains unclear, the...
antibiotics administered in this clinical case also included *G. adiacens* in their antimicrobial spectrum.

*Bacillus* species including *B. licheniformis* are able to produce biofilms (Banerjee *et al.*, 1988) and the activity of various antibiotics, e.g. vancomycin, is known to be reduced in biofilms due to insufficient penetration into the biofilm matrix (Kassar *et al.*, 2009). The combination of the known ability of *B. licheniformis* to form spores and to produce biofilms with, as shown in this study, the ability to exist as the SCV phenotype makes the persistent and recurrent infection even more possible and supports the concept that a clinical cure requires removal of all foreign materials, which has been stressed by other authors for this and other *Bacillus* species (Blue *et al.*, 1995; Quan *et al.*, 2000; Santini *et al.*, 1995).

Although the main mechanism of cardiac-device-related infective endocarditis is contamination by local bacterial flora at the time of device implantation, another possible mechanism includes haematogenous seeding from a distant focus of infection (Habib *et al.*, 2009). In this case study it was difficult to elucidate the origin of infection, since 3 years had elapsed since the reimplantation of the pacemaker and the first episode of *B. licheniformis* bacteraemia. However, the recurrent lung infections which preceded this bacteraemia were, in retrospect, most probably a consequence and manifestation of pulmonary embolism, even if a TOE could not reveal vegetations on PLs or right heart valves at that time. Pulmonary embolism is a frequent complication of PL-related IE (Habib *et al.*, 2009). Interestingly, in a reported case of *B. licheniformis* PL infection, a TOE revealed no vegetation on PLs or heart valves, although lead tip and a number of blood cultures yielded *B. licheniformis* (Quan *et al.*, 2000). Removal of the cardiac device is recommended in all cases of proven cardiac-device-related IE and should also be considered if cardiac-device-related IE is only suspected in the case of occult infection without any other apparent source (Baddour *et al.*, 2010; Habib *et al.*, 2009). This report provides evidence that consideration to remove pacemakers should be especially strong if *B. licheniformis* is isolated from blood cultures in pacemaker patients with infections that are difficult to diagnose.

*B. licheniformis* can cause recurrent foreign-body-related infections due to its ability to form biofilms. As shown here for the first time, *B. licheniformis* isolates displaying the SCV phenotype must not be disregarded and careful microbiological examination is mandatory to confirm species affiliation and involvement of those isolates as causative agents.

**Acknowledgements**

We are thankful to B. Grünastel for excellent technical assistance.

**References**


