

## *Bacillus licheniformis* Prosthetic Aortic Valve Endocarditis

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**A 73-year-old man developed an acute prosthetic aortic valve dehiscence for which emergent operation was undertaken. The intraoperative evidence of an aortic annular disruption and of a subannular abscess led to the hypothesis that an endocarditis process was involved. The aortic valve was replaced with a stentless porcine bioprosthesis. Cultures taken intraoperatively from the aortic area had a pure growth of aerobic, spore-forming, gram-positive bacilli identified as *Bacillus licheniformis*. The patient responded to specific antibiotic therapy with no relapse at a 20-month follow-up. The potentiality of *B. licheniformis* as a pathogen should be reconsidered.**

With the exception of *Bacillus anthracis* infections, serious infections caused by aerobic, spore-forming, gram-positive *Bacillus* species are rare (5). Only a few cases have been reported previously, and these have often occurred in conjunction with the host's immunological incompetence, associated with operative procedures, wounds and burns, hemodialysis, and food poisoning, and in a parenteral drug abuser (5, 11). We describe here a unique case of life-threatening *B. licheniformis* endocarditis occurring on a prosthetic aortic valve, for which reoperation was successfully undertaken. To the best of our knowledge, this is the first reported case of endocarditis due to this etiology in an immunologically normal patient.

A 73-year-old white male under medical treatment for arterial hypertension and type II diabetes was admitted to the University of Verona Medical School hospital with acute pulmonary edema. He had undergone 12 years before an aortic valve replacement (25-mm-diameter mechanical prosthesis; St. Jude Medical Inc., St. Paul, Minn.) in association with an epicardial pacemaker implantation (C2141; Vitatron Medical B.V., Dieren, The Netherlands) for the treatment of a calcified aortic valve with a complete atrio-ventricular block.

On admission, the patient presented with dyspnea at rest and leg edema. His blood pressure was 100/45 mm Hg, and his body temperature was 37.4°C. The hematocrit was 40% with a hemoglobin level of 13.6 g/100 ml. The leukocyte count was 9,150/mm<sup>3</sup> with 86.9% neutrophils.

The erythrocyte sedimentation rate and the C-reactive protein level were moderately elevated. A grade IV/VI blowing diastolic murmur was audible in the aortic area. A chest roentgenogram revealed a moderate cardiomegaly (cardiothoracic ratio, 0.6%) and evidence of severe pulmonary edema. Two-dimensional transesophageal echocardiography showed severe aortic valve regurgitation due to an extensive periprosthetic leak associated with diminished left-ventricular function. Cardiac catheterization confirmed the diagnosis and revealed normal coronary arteries. The patient was then taken to the operating room, where, under general anesthesia and with total cardiopulmonary bypass, he underwent removal of the previously implanted artificial aortic valve, detached for about one-

third of its circumference. After removal of the valve, a limited abscess was noted underneath the aortic ring between the right and noncoronary cusps. Therefore, on the basis of the hypothesis that the prosthetic valve detachment could be due to an

TABLE 1. Growth and biochemical characteristics of *B. licheniformis*

Test or characteristic <sup>a</sup>	Result <sup>b</sup>
Gram reaction.....	+
Catalase production.....	+
Motility.....	+
Anaerobic growth.....	+
Growth at 50°C.....	+
Growth at 60°C.....	-
Growth at pH 5.7.....	+
Growth in 7% NaCl.....	+
Growth in 0.001% lysozyme.....	-
Egg yolk reaction.....	-
Lipid globules in protoplasm.....	-
Spore type.....	Ellipsoidal, central, no swelling of sporangium
Esculin.....	+
ONPG.....	+
ADH.....	+
LDC.....	-
ODC.....	-
Urease.....	-
TDA.....	-
Citrate utilization.....	±
H <sub>2</sub> S production.....	-
Indole production.....	-
Voges-Proskauer.....	+
Gelatin decomposition.....	+
Nitrate reduction.....	+
Acid from:	
Starch, glycerol, ribose, D-xylose, galactose, glucose, fructose, mannose, inositol, mannitol, sucrose, arbutin, cellobiose, maltose.....	+
Sorbitol, lactose, inulin, melibiose, raffinose, D-tagatose.....	±
Erythritol, dulcitol, xylitol, gluconate.....	-

<sup>a</sup> Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; ADH, arginine dehydrolyase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; TDA, tryptophan deaminase.

<sup>b</sup> Results are given as follows: +, ≥81% positive reactions; ±, 20 to 80% positive reactions; -, ≤19% positive reactions (48-h incubation at 37°C).

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TABLE 2. Growth characteristics of selected *Bacillus* species

Test or characteristic	Result <sup>a</sup> for species <sup>b</sup> :											
	BC1	BC2	BAN	BBR	BAL	BCO	BPU	BLI	BSU	BI1	BI2	BMA
Gram reaction	+	+	+	±	±	+	+	+	+	±	±	±
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	-	-	+	+	+	+	+	+	±	±	+
Growth:												
Anaerobic	+	+	+	-	+	+	-	+	-	±	±	+
At 50°C	-	-	-	+	-	+	+	+	+	+	+	+
At 60°C	-	-	-	±	-	±	-	-	-	-	-	-
At pH 5.7	+	+	+	±	-	+	-	+	+	±	±	+
In 7% NaCl	+	+	+	-	-	-	+	+	+	±	±	-
Spores:												
Ellipsoidal	+	+	+	+	+	+	+	+	+	+	+	+
Spherical	-	-	-	-	-	-	-	-	-	-	-	-
Central	+	+	+	±	±	±	+	+	+	±	±	-
Terminal	-	-	-	±	±	±	-	-	-	±	±	+
Swelling sporangium	-	-	-	+	+	±	-	-	-	+	+	+

<sup>a</sup> Results are given as follows: +, ≥81% positive reactions; ±, 20 to 80% positive reactions; -, ≤19% positive reactions (48-h incubation at 37°C).

<sup>b</sup> Abbreviations: BC1, *B. cereus* 1; BC2, *B. cereus* 2; BAN, *B. anthracis*; BBR, *B. brevis*; BAL, *B. alvei*; BCO, *B. coagulans*; BPU, *B. pumilus*; BLI, *B. licheniformis*; BSU, *B. subtilis*; BI1, *B. circulans* 1; BI2, *B. circulans* 2; BMA, *B. macerans*.

infective etiology, the explanted prosthesis and samples from the abscess were taken for cultures. After an extensive debridement of the aortic root and irrigation with antiseptic solution, a 23-mm-diameter stentless porcine aortic valve (Biocor Indu-

stria e Pesquisa Ltda, Belo Horizonte, MG, Brazil) was implanted. Patient recovery in the intensive care unit was uneventful.

Tissue cultures from the explanted prosthesis, homogenized

TABLE 3. Biochemical characteristics of selected *Bacillus* species

Test or characteristic <sup>a</sup>	Result <sup>b</sup> for species <sup>c</sup> :											
	BC1	BC2	BAN	BBR	BAL	BCO	BPU	BLI	BSU	BI1	BI2	BMA
Glycerol	±	±	-	±	+	±	+	+	+	±	±	+
Ribose	+	+	+	±	+	-	+	+	+	±	+	+
D-Xylose	-	-	-	-	-	-	±	+	±	+	+	+
Galactose	-	±	-	-	±	+	+	+	±	+	+	+
Glucose	+	+	+	-	+	+	+	+	+	+	+	+
Fructose	+	+	+	±	+	+	+	+	+	+	±	+
Mannose	-	-	-	-	±	+	+	+	+	+	+	+
Sorbose	-	-	-	-	±	-	-	±	-	-	-	-
Dulcitol	-	-	-	-	±	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	+	+	+	-	±
Mannitol	-	-	-	-	-	-	+	+	+	+	±	+
Arbutin	+	±	-	-	±	±	+	+	+	+	+	+
Esculin	+	+	+	±	+	±	+	+	+	+	+	+
Cellobiose	±	-	-	-	-	±	+	+	+	+	+	+
Maltose	+	+	+	-	+	+	±	+	+	+	+	+
Lactose	-	±	-	-	-	±	±	±	-	+	+	+
Melibiose	-	-	-	-	±	+	-	±	±	+	+	+
Sucrose	±	+	+	-	±	±	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	±	+	+	±	+
Raffinose	-	-	-	-	±	±	-	±	±	+	+	+
Starch	+	-	+	-	±	+	-	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	-	-	+	-	-
D-Tagatose	-	-	-	-	-	-	+	±	-	-	-	±
Gluconate	-	-	-	-	-	±	-	-	-	+	±	±
ONPG	-	-	-	±	+	+	+	+	+	+	±	+
ADH	+	-	-	-	-	-	-	+	-	-	-	±
Citrate	±	+	-	±	-	-	+	±	+	±	-	±
Urease	-	-	-	-	±	-	-	-	-	-	-	-
Indole	-	-	-	-	±	-	-	-	-	-	-	-
VP	±	+	+	±	+	+	+	+	+	±	-	±
Gelatin	+	+	+	±	+	±	+	+	+	-	-	±
Nitrate	±	+	+	±	±	±	±	+	+	±	-	±

<sup>a</sup> Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; ADH, arginine dehydrolase; VP, Voges-Proskauer.

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TABLE 4. Types and numbers of cases of reported infections caused by *B. licheniformis*

Source, yr	Type of infection	Associated condition(s)	No. of cases
Peloux, 1976	Bacteremia	Pregnancy (acute fibrinolysis)	1
Sugar, 1977	Bacteremia, peritonitis	Volvulus, perforation of upper small bowel	1
Jephcott, 1977	Diarrheal illness	Food poisoning	Undetermined
Tabbara, 1979	Corneal ulcer	Eye injury	1
Young, 1982	Ventriculitis	Meningioma	1
Hardy, 1986	Septicemia	Arteriography	1
Jones, 1992	Cerebral abscess	Penetrating orbital injury	1

in a sealed plastic bag by using a Stomacher Lab Blender (Spiral Systems Instruments, Inc., Bethesda, Md.), and cultures from the abscess cavity samples had a pure growth of aerobic, spore-forming, gram-positive bacilli that were identified on the basis of specific morphological and biochemical reactions as *B. licheniformis*.

The specimens were plated on blood agar (BA), Columbia colistin-nalidixic acid blood agar (CNA), chocolate agar (CA), MacConkey agar, and Sabouraud agar and incubated for 24 h in air at 37°C. They were also plated on Schaedler agar, Schaedler agar plus vancomycin and gentamicin, and Columbia CNA agar and incubated for 48 h at 37°C in an anaerobic atmosphere; an enrichment of brain heart broth was added after an incubation of 24 h.

From the BA, CNA, CA, and Schaedler (without antibiotics) agar plates, a gram-positive, spore-bearing (central spores produced), catalase- and indophenol oxidase-positive strain with opaque rough-surface colonies was recovered; the growth was hairlike, and slime accumulated on the colonies in the form of mounds and lobes.

According to macroscopical, microscopical, and biochemical characteristics, the strain was presumptively identified as a *Bacillus* sp. (8). It was definitively identified by using the API 50 CHB system (API Laboratory Products, Basingstoke, Hampshire, United Kingdom) susceptibility test kit according to the guidelines set out by the National Committee for Clinical Laboratory Standards, and breakpoints were also determined. The definitive identification was *B. licheniformis* (Tables 1 to 3).

The organism proved to be susceptible to cephalothin, gentamicin, clindamycin, vancomycin, and trimethoprim-sulfamethoxazole; it was resistant to penicillin, ampicillin, methicillin, tetracycline, and erythromycin. On the basis of susceptibilities, an antibiotic regimen of 1 g of cefazolin sodium every 6 h was initiated and continued for 6 weeks (outpatient clinic). Serial postoperative blood cultures were negative. Patient recovery was uneventful, with freedom from recurrent endocarditis, reoperation, and valve-related complications at a 20-month follow-up.

**Comment.** Given the ubiquitous distribution of *Bacillus* species in the environment, it is not surprising that these organisms are often encountered in diagnostic laboratory cultures, where, with the exception of *B. anthracis*, they are usually regarded as nonpathogenic or as simple contaminants (5). More recently, however, other species of *Bacillus* have been increasingly recognized as important pathogens in susceptible hosts (4). *B. licheniformis* in particular has been responsible for cases of bacteremia, septicemia, and peritonitis (2, 6, 9, 11), food-poisoning syndrome (3, 11); ophthalmitis (10); ventriculitis (12); and cerebral abscess (4) (Table 4).

The case presented here, to the best of our knowledge, is the first report of prosthetic aortic valve endocarditis due to this etiology in a patient with no known immunological defect, as

determined by a medical history of a lack of recurrent infections and negative laboratory evaluation of host defense status (complete blood count with differential smear, serum immunoglobulin levels, quantification of blood mononuclear cell populations, and complement and phagocyte function). Endocarditis due to *B. subtilis* and *B. cereus*, often in drug abusers, has been described previously (1, 7).

Prosthetic aortic valve endocarditis is a dreadful complication of heart valve surgery. In the patient described here, a rapidly progressive heart failure developed as a consequence of an acute mechanical prosthetic valve dehiscence which required emergent surgical intervention. The intraoperative evidence of an aortic annular disruption and of a subannular abscess led to the hypothesis that an endocarditis process was involved. Cultures taken intraoperatively from the abscess cavity and mechanical valve grew *B. licheniformis*. In this case, it is unlikely that the *Bacillus* sp. was a contaminant, since it grew on all culture plates seeded, other organisms were not isolated, and the patient responded to specific antibiotic therapy with no relapse at a 20-month follow-up.

Organisms of the genus *Bacillus* are aerobic, spore-forming, gram-positive rods which are found commonly in air, soil, dust, hay, milk, water, and wool. The possible source of infection in our patient has been investigated retrospectively, but no apparently significant underlying conditions or events in the patient's medical history were recorded (including intravenous drug abuse, sickle cell disease, trauma, foreign bodies and invasive medical procedures, malignancy, neutropenia, corticosteroid therapy, and AIDS). Thus, the source of contamination in the presented case still remains unclear.

In conclusion, our experience emphasizes another aspect of the potentiality of *B. licheniformis* as a pathogen. Its benignity as a conventional microorganism in immunologically competent patients should be reconsidered.

#### REFERENCES

- Craig, C. P., W. S. Lee, and M. S. Ho. 1974. *Bacillus cereus* endocarditis in an addict. *Ann. Intern. Med.* **80**:418-419.
- Hardy, A., J. M. Feger, Y. Piemont, and P. Bareiss. 1986. Etat septique apres arteriographie. Du role d'un germe reputé non pathogene: *Bacillus Licheniformis*. *J. Med. (Strasbourg)* **17**:18-19.
- Jephcott, A. E., B. W. Barton, R. J. Gilbert, and C. W. Shearer. 1977. An unusual outbreak of food-poisoning associated with meals-on-wheels. *Lancet* **ii**:129-130.
- Jones, B. L., M. F. Hanson, and N. A. Logan. 1992. Isolation of *Bacillus licheniformis* from a brain abscess following a penetrating orbital injury. *J. Infect.* **24**:103-104.
- Logan, N. A. 1988. *Bacillus* species of medical and veterinary importance. *J. Med. Microbiol.* **25**:157-165.
- Peloux, Y., C. Charrel-Taranger, and F. Gouin. 1976. Nouvelle affection opportuniste a *Bacillus*: un cas de bacteriemie a *Bacillus Licheniformis*. *Pathol. Biol.* **24**:97-98.
- Reller, L. B. 1973. Endocarditis caused by *Bacillus subtilis*. *Am. J. Clin. Pathol.* **60**:714-718.
- Sneath, P. H. A. 1986. Endospore-forming gram-positive rods and cocci, p. 1104-1207. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.),

- Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore.
9. **Sugar, A. M., and R. V. McCloskey.** 1977. *Bacillus licheniformis* sepsis. JAMA **238**:1180-1181.
  10. **Tabbara, K. F., and N. Tarabay.** 1979. *Bacillus licheniformis* corneal ulcer. Am. J. Ophthalmol. **87**:717-719.
  11. **Tuazon, C. U., H. W. Murray, C. Levy, M. N. Solny, J. A. Curtin, and J. N. Sheagren.** 1979. Serious infections from *Bacillus* species. JAMA **241**: 1137-1140.
  12. **Young, R. F., R. N. Yoshimori, D. L. Murray, and P. J. Chou.** 1982. Post-operative neurosurgical infections due to *Bacillus* species. Surg. Neurol. **18**:271-273.