Case Report

Subacute infective endocarditis caused by *Bacillus cereus* in a patient with Systemic Lupus Erythematosus

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Abstract

A rare and difficult to diagnose case of subacute infective endocarditis caused by *Bacillus cereus* in a patient with systemic lupus erythematosus and Libman-Sacks endocarditis has been reported. Our aim is to highlight the importance of molecular methods such as MALDI-TOF and PCR to explain clinical and epidemiological issues about infections caused by unusual pathogen.

Key words: Infective endocarditis; *Bacillus cereus*; systemic lupus erythematosus; polymerase chain reaction; MALDI-TOF.

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Introduction

*Bacillus cereus sensu stricto* (s.s.) is a member of the Bacillus cereus complex (BCC), a group of Gram-positive endospore-forming rod-shaped bacilli present worldwide, commonly found in soil and water. *B. cereus s.s.* is most frequently associated with non-lethal foodborne gastrointestinal diseases [1]. However, in recent years, *B. cereus s.s.* has emerged as an important pathogen causing device-related infections, especially in immunocompromised patients. In this context, *B. cereus s.s.* complicated bloodstream infections such as infective endocarditis (IE) associated with central lines, pacemakers and prosthetic valves have been reported [2]. These infections can be difficult to treat due to the high beta-lactam resistance rates and the agent’s ability to form biofilms [3,4]. Therefore, the rising number of cases of severe extra-gastrointestinal infections caused by *B. cereus* is of growing concern to clinicians and infection control specialists.

Species identification of BCC members by using traditional methods is challenging [5]. Biochemical identification systems routinely used in microbiological laboratories are not usually able to discriminate *B. cereus s.s.* from highly pathogenic species such as *Bacillus anthracis*. Moreover, the characterization of BCC members at species level according to 16S rRNA gene sequence is not appropriate given the very high sequence similarities of this gene observed in *B. cereus* and *B. anthracis*, with a sequence homology of ≥ 99%. The identification of *B. cereus* with Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been successfully performed. MALDI-TOF is a fast, reliable and relatively inexpensive technique widely applied to the identification of important clinical pathogens [6].

BBC species, mainly *B. cereus s.s.* and *B. thuringiensis*, are well known for their ability to produce several virulence factors such as toxins, hemolysins, and enzymes. BBC enterotoxins, hemolysin BL (HBL) and nonhemolytic enterotoxin (NHE) are considered the primary factors that can cause food poisoning [7]. Cytotoxin K (CytK), a simple protein with necrotic and hemolytic activity, also contributes to intoxication by *B. cereus* and can be found as two variants: CytK1 and CytK2. Enterotoxin FM (EntFM) has also been reported in *B. cereus* and is cytotoxic to Vero cells. Hemolysins II (HlyII) and III (HlyIII) and phospholipases produced by *B. cereus* are...
also associated with extra-gastrointestinal infections [8]. *B. cereus* phosphatidyleholine-specific phospholipase C and sphingomyelinase (SPH) form a membrane-disrupting complex known as cereolysin AB [9].

Recently, a guide to utilization of the microbiology laboratory for diagnosis of infectious diseases was updated by the Infectious Diseases Society of America and the American Society for Microbiology [10]. In this context, the role of MALDI-TOF and molecular amplification methods to confirm *B. cereus* infection and explain its source as well as clinical presentation are discussed here. In this report, a rare case of subacute/chronic IE due to *B. cereus* s.s. associated with Libman-Sacks endocarditis in a patient with systemic lupus erythematosus (SLE) is presented.

**Methods**

Patient data was collected by medical record review. Microorganism growth and identification of blood samples were performed by BD Bactec™ and BD Phoenix™ (Becton, Dickinson and Company, New Jersey, USA) automated systems, respectively. Optical microscopy was performed to confirm the absence of protein crystals inside the sporangia and to confirm the *B. cereus* s.s. identification. Five of six *B. cereus* isolates obtained from blood samples during the microbiological investigation were available for additional analysis. Identification of these isolates confirmed by MALDI-TOF MS, using the Maldi Biotyper platform (Bruker Daltonics, Bremen, Germany). Polymerase chain reaction (PCR) was performed for detection of toxin encoding genes such as *hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, entFM and ces, [11], cytK-2 [12], and other virulence factors such as *hlyII*, *hlyIII*, *piplc*, *pcpl*, and *sph* [13]. Genetic similarity among the isolates was evaluated by repetitive extragenic palindromic sequence-based PCR analysis (Rep-PCR) as previously described [14]. Minimal Inhibitory Concentration (MIC) to vancomycin, gentamicin and tetracycline were determined by E-TEST® (bioMérieux, Marcy-l'Étoile, France) according to the Clinical and Laboratory Standards Institute for Potential Bacterial Agents of Bioterrorism [15]. This study approved by The Ethics Committee of Hospital Universitário Antonio Pedro of Universidade Federal Fluminense (HUAP), number: 32570 (CAAE 02759912.9.0000.5243).

**Case report**

On day 1, a 30-year-old woman with end-stage renal disease caused by lupus nephritis, on haemodialysis through long-term central venous catheter (LT-CVC), was hospitalized with fever, palpitations, dyspnea, pleuritic pain, worsening of polyarthralgia and malar rash. The hypothesis of myopericarditis associated with SLE activity raised and pulse therapy with methylprednisolone was initiated. On day 5, transthoracic echocardiogram (TT-ECO) showed calcified echogenic lesions of the mitral valve, causing both regurgitation and stenosis, and a suspected small mitral vegetation. Such TT-ECO findings raised the hypothesis of Libman-Sacks endocarditis or, less likely, a superadded infective endocarditis (IE). To investigated IE, a set of three peripheral blood samples was cultured. On day 7, after a week of immunosuppressive therapy without antibiotic therapy, the patient was asymptomatic and was discharged with prescription of mycophenolate and prednisone with blood culture results pending.

On day 54, the patient was hospitalized again due to fever (Axillary temperature: 38.1 °C) with no other symptoms of infection. Physical examination revealed systolic mitral murmur (3+/6) without peripheral stigmata of IE. Hematological parameters showed normocytic normochromic anemia without leukocytosis and elevation of serum C-reactive protein level (CRP: 3.44 mg/dL; Reference value < 0.8 mg/dL). Additionally, one of the three peripheral blood samples cultured during the previous hospitalization showed growth of *B. cereus*. Four additional blood samples (three peripheral and one from LT-CVC) were collected for culture and tranesophageal echocardiography (TEE) was performed. The four blood samples were positive for *B. cereus* and the main finding on TEE was a small image in a posterior leaflet of mitral valve, suggesting vegetation. On day 57, vancomycin was initiated for treatment of subacute IE caused by *B. cereus*. After seven days of antimicrobial therapy, additional blood cultures of samples obtained from peripheral vein and LT-CVC were tested to evaluate bacteremia persistence, it showed *B. cereus* growth only in the samples obtained from LT-CVC, suggesting catheter–related infection. Consequently, the LT-CVC removed. After six weeks of therapy with vancomycin, the patient was asymptomatic with decrease of CRP serum level. On day 105, the patient was discharged with a new TEE showing absence of vegetation.

Additional microbiological characterization of the five bacterial isolates performed with MALDI-TOF (Bruker Daltonics, Bremen, Germany), showed score values of 2.129-2.257, confirming the identification of *B. cereus*. Analysis of toxins and virulence genes by PCR showed that all isolates were positive for all genes.
investigated, except \textit{hlyII}, and shared the same virulence (\textit{hlyIII}, \textit{piplc}, \textit{pcpl}, and \textit{spf}) and toxigenic (\textit{hblC, hblD, nheA, nheB, nheC, entFM, ces} and \textit{cytK-2}) profiles. Molecular typing by Rep-PCR showed that all isolates shared the same fingerprint pattern. MICs determined by E-test ranged from 0.38 to 3.0 \(\mu\text{g/mL}\) for vancomycin, from 0.023 to 1.0 \(\mu\text{g/mL}\) for tetracycline, and from 0.25 to 0.64 \(\mu\text{g/mL}\) for gentamicin.

\section*{Discussion}

In this report, a rare and difficult to diagnose case of subacute/chronic IE due to \textit{B. cereus} in a patient with SLE is described. The use of molecular methods such as MALDI-TOF, gene specific PCR and Rep-PCR were essential to determine \textit{B. cereus s.s.} as the etiologic agent of IE, to understand its clinical presentation and to elucidate the extra-gastrointestinal source of infection, respectively. Therefore, these approaches contributed greatly to elucidate relevant epidemiological and clinical issues.

The differential diagnosis between IE vegetations and other images present in SLE such as Libman-Sacks endocarditis can be very difficult. In the present case, the diagnosis of subacute/chronic IE was supported by the presence of valve vegetation associated with \textit{B. cereus s.s.} growth in blood samples cultured within an interval of about eight weeks [16]. MALDI-TOF MS, a rapid, accurate, and relatively inexpensive technique was essential to confirm the initial microbiological identification of \textit{B. cereus} done by BD Phoenix™ automated systems. The isolation of \textit{B. cereus s.s.} belonging to a single Rep-PCR genotype in blood samples collected from the LT-CVC and peripheral vein in a patient without clinical manifestations of gastrointestinal disease supported the diagnosis of catheter-related bloodstream infection with IE [17]. In addition, the same \textit{B. cereus s.s.} strain was found in the peripheral blood and LT-CVC samples collected over the course of the infection, suggesting catheter colonization. Ikram \textit{et al.} (2019) recently described two cases of CVC-related \textit{B. cereus} in cardiotic patients. Genetic analysis of the two strains isolated showed that they were closely related to other strains in the emetic cluster [18]. Interestingly, the \textit{B. cereus s.s.} strain isolated in this study harboured the \textit{ces} gene, characteristic of strains belonging to the emetic cluster. These findings point to an opportunistic \textit{B. cereus s.s.} strain causing infection by taking advantage of host’s risk factors such as the presence of intravascular device, previous endocardium Libman-Sacks lesions, and immunosuppressive therapy. In addition, this strain did not harbor \textit{hlyII}, known as a good indicator of pathogenicity in \textit{B. cereus s.s} [8]. This virulence gene profile is likely associated with low ability to cause tissue damage and could explain the subacute/chronic clinical presentation as well as the benign outcome of the patient in the present case of IE caused by \textit{B. cereus}. Despite of the benign outcome, the extended hospitalization necessary for the effective treatment of the patient contributed to the burden of infection [19].

Finally, this report highlights the contribution of molecular methods to clarify relevant clinical and epidemiological issues of a difficult to diagnose case of IE caused by an unusual pathogen. Infectious diseases are very dynamic; old pathogens causing new diseases and presenting new forms of transmission emerge frequently. In this setting, the present case points to how different microbiological methods can help to improve medical practices in infectious diseases and better understand their changings.

\section*{References}


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