JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Case Report

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

Rachel Leite Ribeiro[1], Matheus Oliveira Bastos[1], Alec Morse Blanz[1], Jaqueline Abel da Rocha[2], Nathalia Antonio de Oliveira Velasco[1], Andressa Temperini de Oliveira Marre[3], Raiane Cardoso Chamon[1], Leonardo Alves Rusak[4], Adriana Marcos Vivoni[4], Ianick Souto Martins[1]

[1] *Faculty of Medicine, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil*
[2] *Infection Control Section, Hospital Universitário Antonio Pedro, Universidade Federal Fluminense, Niterói, Brazil*
[3] *Institute of Microbiology Paulo de Goés, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[4] *Institute Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil*
[5] *Infection Control Section, Hospital do Câncer I, National Institute of Cancer, Brazil*

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.

J Infect Dev Ctries 2022; 16(4):733-736. doi:10.3855/jidc.15685

(Received 30 July 2021 – Accepted 11 October 2021)

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 seguence 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* phosphatidylcholine-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 transesophageal 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. cereu*s. 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. cereu*s 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 *hlyII*, and shared the same virulence (*hlyIII*, *piplc*, *pcpl*, and *sph*) and toxigenic (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *entFM*, *ces* and *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 µg/mL for vancomycin, from 0.023 to 1.0 µg/mL for tetracycline, and from 0.25 to 0.64 µg/mL for gentamicin.

Discussion

In this report, a rare and difficult to diagnose case of subacute/chronic IE due to *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 *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 *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 *B. cereus* done by BD Phoenix ™ automated systems. The isolation of *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 *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 *et al*. (2019) recently described two cases of CVC-related *B. cereus* in cardiatic patients. Genetic analysis of the two strains isolated showed that they were closely related to other strains in the emetic cluster [18]. Interestingly, the *B. cereus s.s* strain isolated in this study harboured the *ces* gene, characteristic of strains belonging to the emetic cluster. These findings point to an opportunistic *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 *hlyII*, known as a good indicator of pathogenicity in *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 *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.

References

1. Griffiths MW, Schraft H (2017) *Bacillus cereus* food poisoning. In: Foodborne diseases: 3rd edn.
2. Wright WF (2016) Central venous access device-related *Bacillus cereus* endocarditis: A case report and review of the literature. Clin Med Res 14: 109–115.
3. Kuroki R, Kawakami K, Qin L, Kaji C, Watanabe K, Kimura Y, Ishiguro C, Tanimura S, Tsuchiya Y, Hamaguchi I, Sakakura M, Sakabe S, Tsuji K, Inoue M, Watanabe H (2009) Nosocomial bacteremia caused by biofilm-forming *Bacillus cereus* and *Bacillus thuringiensis*. Intern Med 48: 791–796.
4. Veysseyre F, Fourcade C, Lavigne JP, Sotto A (2015) *Bacillus cereus* infection: 57 case patients and a literature review. Med Mal Infect 45: 436–440.
5. Pauker VI, Thoma BR, Grass G, Bleichert P, Hanczaruk M, Zöller L, Zange S (2018) Improved discrimination of *Bacillus anthracis* from closely related species in the *Bacillus cereus* sensu lato group based on matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 56: e01900-17.
6. Ash C, Farrow JA, Dorsch M, Stackebrandt E, Collins MD (1991) Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int J Syst Bacteriol 41: 343-6
7. Senesi S, Ghelardi E (2010) Production, secretion and biological activity of *Bacillus cereus* enterotoxins. Toxins (Basel) 2: 1690-703,
8. Cadot C, Tran SL, Vignaud ML, De Buyser ML, Kolstø AB, Brisabois A, Nguyen-Thé C, Lereclus D, Guinebretière MH, Ramarao N (2010) InhA1, NprA, and HlyII as candidates for markers to differentiate pathogenic from nonpathogenic *Bacillus cereus* strains. J Clin Microbiol 48: 1358-65.
9. Ruiz-Argüello MB, Goñi FM, Alonso A (1998) Phospholipase C hydrolysis of phospholipids in bilayers of mixed lipid compositions. Biochemistry 37: 11621-8.
10. Miller JM, Binnicker MJ, Campbell S, Carroll KC, Chapin KC, Gilligan PH, Gonzalez MD, Jerris RC, Kehl SC, Patel R, Pritt BS, Richter SS, Robinson-Dunn B, Schwartzman JD, Snyder JW, Telford S 3rd, Theel ES, Thomson RB Jr, Weinstein MP,

Yao JD (2018) A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 67: e1-e94.
11. Yang, IC, Shih DY, Huang TP, Huang YP, Wang JY, Pan TM (2005) Establishment of a novel multiplex PCR assay anddetection of toxigenic strains of the species in the *Bacillus cereus* group. J Food Prot 68: 2123–2130.
12. Guinebretiere MH, Fagerlund A, Granum PE, Nguyen-Thé C (2006) Rapid discrimination of cytK-1 and cytK-2 genes in *Bacillus cereus* strains bya novel duplex PCR system. FEMS Microbiol Lett 259: 74–80.
13. Hendriksen NB, Hansen BM & Johansen JE (2006) Occurrence and pathogenic potential of *Bacillus cereus* group bacteria in a sandy loam. Antonie van Leeuwenhoek 89: 239 – 249.
14. Reyes-Ramirez A, Ibarra JE (2005) Fingerprinting of *Bacillus thuringiensis* type strains and isolates by using *Bacillus cereus* group-specific repetitive extragenic palindromic sequence-based PCR analysis. Appl Environ Microbiol 71: 1346–1355.
15. Clinical and Laboratory Standards Institute (CLSI) (2010) Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline, 2nd edn. M45A2E.
16. Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA; American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Stroke Council (2015) Infective endocarditis in adults: Diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation 132: 1435-86.
17. Centers for Disease Control (2017) Central line-associated bloodstream infection and non-central line-associated bloodstream infection. Bloodstream Infection Event, Device-associated Modul BSI.
18. Ikram S, Heikal A, Finke S, Hofgaard A, Rehman Y, Sabri AN, Økstad OA (2019) *Bacillus cereus* biofilm formation on central venous catheters of hospitalised cardiac patients, Biofouling 35: 204-216.
19. Da Rocha JA, Do Valle FM, Da Silva NCZ, de Araújo WN, Martins IS (2017) Disability adjusted life year (DALY) of central-line bloodstream infection (CLABSI) in a university hospital in a developing country, Brazil. Infect Control Hosp Epidemiol 38: 606–609.

Corresponding authors

Professor Ianick Souto Martins, MD, PhD.
Hospital Universitário Antonio Pedro, Universidade Federal Fluminense,
Rua Marques de Paraná 303, Nitérói, RJ, CEP: 24033-900, Brazil.
Phone: 55 21 26299317; 55 21 99824-9340;
Fax: 55 21 24542730
Email address: ianicksm@id.uff.br

Professor Rachel Leite Ribeiro, PhD.
Hospital Universitário Antonio Pedro, Universidade Federal Fluminense,
Rua Marques de Paraná 303, Nitérói, RJ, CEP: 24033-900, Brazil.
Phone: 55 21 984952189
Fax: 55 21 26299104
Email address: rachelribeiro@id.uff.br

Conflict of interests: No conflict of interests is declared.