Introduction

The genus Clostridium gathers anaerobic Gram-positive rods that are capable of forming endospores. The majority of members of this genus are strict anaerobes, but there are considerable species variations with respect to oxygen toxicity (Krieg et al., 2010). There are at least 150 species described, most of them are saprophytic and less than 20 species are related to human diseases (Shu et al., 2008). Although members of the genus are usually found as microbiota of the human intestinal tract, their disease spectra in humans are considered to be broad (Hannett et al., 2014).

Clostridium baratii is an obligately anaerobic bacterium that is rarely encountered as causing human infections (Hannett et al., 2014). In 1985 the first report of botulism neurotoxin production by a strain of this organism was made (Hall et al., 1985). Hannett et al. (2014) have described two cases of F botulism infection in elderly patients, 79- and 68-years-old, both with history of diplopia and weakness. Since both cases were clustered together in time and geography, molecular typing was performed and proved that they were unrelated genetically. In 2015, three cases of food-borne botulism by the consumption of Bolognese sauce were reported in France (Tréhard et al., 2016). In this case, strains were isolated from stools and the authors proved the meat used to make the sauce to be the contaminant.
C. baratii producing type F neurotoxin is a potential human pathogen and some severe cases, such as a 3-year-old boy with Kawasaki syndrome (Iaria et al., 2007), a lung abscess in a patient with an invasive pulmonary aspergillosis (Shu et al., 2008), and a liver abscess in a healthy adult (Huang et al., 2012) have been reported over the years. Here we report a case of pneumonia by a non-toxigenic strain of C. baratii in an Alzheimer 70-year-old male with sepsis in Rio de Janeiro, Brazil.

**Case report**

In 2014, a 70-year-old man was admitted to a private hospital with complaints of asthenia and night fever. Empirical treatment of urinary tract infection was in progress with ciprofloxacin 500 mg twice a day outpatient, with no clinical answer. The patient also had previous diagnoses of chronic kidney disease, benign prostatic hyperplasia, chronic ischemic stroke and Alzheimer’s disease.

On admission, pulmonary and abdominal computed tomography (CT) scans were performed. Left inferior lobe consolidation and a small pleural bilateral effusion (Fig. 1) were identified. There was also a fecaloma, promoting abdominal distention.

The primary diagnosis was lung sepsis, and the patient was admitted to an intensive care unit (ICU). Urinalysis, urine culture, haemogram, haemoculture and C-reactive protein (CRP) dosage were made. A few hours after ICU admission, empiric antibiotic therapy was started. The initial regimen of antibiotic was piperacillin-tazobactam 3.375 g intravenously three times a day. After seven days on this regimen, the patient still presented a high leucocytes count and increasing levels of CRP. Unfortunately, both blood and urine cultures were negative. Facing this clinical scenario, the medical team chose to change antibiotic and perform another pulmonary CT scan. Empirically, meropenem 1 g twice a day was started to replace piperacillin-tazobactam. The latest pulmonary CT scan showed a worsening of pleural effusion and intense pleural bleeding in the left lung. Despite the change of the antibiotic regimen, and its use for a week, clinical improvement was not observed. The patient still had a fever, respiratory distress and haemodynamic instability. At this point, another diagnostic measure was attempted: a surgical pleuroscopy. During this procedure, both pleural effusion and its fragments were obtained for microbiology screening.

Materials obtained by pleuroscopy were inoculated on blood agar plates (5 % desfibrinated sheep blood), chocolate agar with Vitox (PlastLabor) and in a BD BACTEC anaerobic test bottle (Becton Dickinson). All plates were incubated for 48 h at 37 °C, under anaerobic and capnophilic atmosphere. The blood culture was positive seven days after inoculation. Gram stain of this culture revealed the presence of Gram-positive spore-forming rods. The flask was sent to the Laboratory of Anaerobes Biology at Universidade Federal do Rio de Janeiro (UFRJ), where it was inoculated on 5 % sheep blood agar plates supplemented with 5 mg l⁻¹ haemin and 1 mg ml⁻¹ menadione. The plates were incubated in the COY glove box (Coy Laboratory) and revealed translucid, β-haemolytic, smooth, circular, yellow-pigmented colonies after incubation for 48 h. Two analyses were conducted for the identification of the sample: (i) phenotypical tests (Table 1) (Lousiemies-Somier et al., 2002) and (ii) mass spectrometry (MS) by MALDI-TOF BioTyper System (Bruker Daltonics). Tests indicated a strain of C. baratii. Additionally antimicrobial susceptibility patterns and MIC values were determined by the ETEST strips (bioMérieux) and were used to breakpoints standardized by the Clinical and Laboratory Standard Institute (CLSI, 2014). For quality control, clinical isolates, namely Bacteroides fragilis ATCC 25285 and Staphylococcus aureus ATCC 29213, were used in parallel for each incubation. The following antibiotics were tested: vancomycin (MIC 0.38 µg ml⁻¹ – sensitive), metronidazole (MIC 0.30 µg ml⁻¹ – sensitive), clindamycin (MIC 0.38 µg ml⁻¹ – sensitive), ertapenem (MIC 0.006 µg ml⁻¹ – sensitive) and imipenem (MIC 0.094 µg ml⁻¹ – sensitive).

Genotypic characterization was done in the Veterinary School of the Universidade Federal de Minas Gerais. Total bacterial DNA was extracted using the commercial kit QIAamp DNA mini kit (QIagen) according to the manufacturer’s instructions. Amplification and sequencing of the 16S rRNA gene were performed according to a previously described method (Fox et al., 2011). PCR products were purified using a Wizard PCR Preps DNA purification system (Promega) and sequenced using forward and reverse primers. Sequencing reactions were performed using a BigDyeTerminator v3.1 cycle sequencing kit (ThermoFisher) and run on an ABI 3730XL genetic analyzer (ThermoFisher). The 16S rRNA gene sequences were compared with

---

**Fig. 1.** Computed tomography of the abdomen showing a mass with fluid-retaining in the central and left cavity of the lung, and thick irregular wall over the right and left lung (LP). A dense mass is shown in the center of right lung (*).
Table 1. Characteristics of Nagler-positive species of the genus Clostridium

| Species       | C. perfringens† | C. baratii‡ | C. bifermentans† | C. sordelli† |
|---------------|-----------------|-------------|-----------------|-------------|
| Indole        | –               | –           | +               | +           |
| Reverse-CAMP test | +         | –           | –               | –           |
| Fermentation of: |                |            |                 |             |
| Mannose       | –               | +           | –               | –           |
| Xylose        | –               | +           | –               | –           |
| Arabinose     | –               | +           | –               | –           |
| Glucose       | +               | +           | +               | +           |

*C. baratii phenotype tests performed in this study.
†Characteristics of Gram-positive spore-forming bacilli according to the Wadsworth Anaerobic Bacteriology Manual, 5th edn.

those of reference strains in the GenBank database of the National Center for Biotechnology Information (http://ncbi.nlm.nih.gov) using the BLASTN computational tool and nucleotide sequence identity ≥98% was used as the criterion for species identification. Together, all tests showed that the organism was C. baratii. The detection of botulinum toxin using PCR (De Medici et al., 2009) and bioassay in mice (Sebald & Petit, 1997) was negative.

After diagnosis, metronidazole 500 mg three times a day intravenously was started and within 24 h the patient had no more fever. One week later, chest radiographs and clinical symptoms had improved.

Discussion

Few cases of infection due to C. baratii have been previously described. The first case reported was a bacteraemia by C. baratii associated with Kawasaki syndrome in a 3-year-old child (Iaria et al., 2007). Shu et al. (2008) described a lung abscess superimposed on invasive pulmonary aspergillosis in a 47-year-old man previously diagnosed with myelodysplastic syndrome. Huang et al. (2012) isolated C. baratii in a liver abscess caused by a cholecystitis empyematomous in a healthy adult. Recently, two cases of adult botulism caused by botulinum neurotoxin-producing C. baratii were reported in patients from the same geographical region. The pathophysiology hypothesis accepted in both cases was intestinal toxemia-related disease (Hannett et al., 2014).

Severe pneumonia described here is a clinical manifestation never before related to C. baratii. Moreover, diseases caused by Clostridium are usually life-threatening conditions, making imperative the prompt diagnosis for successful treatment (Bischoff, 2012). However, the low suspicion of both clinicians and bacteriologists in Brazil concerning commensal micro-organisms as responsible for infection hampers diagnosis. Furthermore, specifically in the case of uncommon micro-organisms, besides the sampling and proper preparation of the patient’s materials, the diagnostic strategy should apply specific and effective methods (Ngo et al., 2013; Park et al., 2009; Tsybuliak & Epifanov, 2009). In our report, at the first medical approach, although samples had been collected, the identification attempt failed. Probably the sampling and/or material preparation were inappropriate. Only after site-specific sampling (pleural effusion and tissue fragments collected by pleuroscopy) and proper preparation of the materials, was isolation of rod-shaped anaerobic Gram-positive cultures possible.

In this current reported case, we isolated a C. baratii non-toxigenic strain but it still caused a severe pulmonary infection. The patient was an Alzheimer 70-year-old male with several complications and probably underwent therapies or procedures that could have perturbed the normal flora and led to colonization of C. baratii. Even though this micro-organism is usually related to intestinal toxemia and infant botulism, in this patient it could have led to a pulmonary infection. This scenario highlights the importance and possible role of commensal anaerobes in human diseases, reinforcing the need for appropriate anaerobic research on biological samples.

Acknowledgements

We grateful to Joaquim Santos and Semiramis Costa from Departamento de Microbiologia Médica, IMPG, UFRJ for technical support and practitioners of the MALDI-TOF mass spectrometry at UFRJ. We are also thankful to FAPERJ, CNPq and CAPES for their financial support.

References

Bischoff, A. (2012). [Clostridium infections. Lethal toxins: what chances are there?]. MMW Fortschr Med 154, 26 (In German).

CLSI (2014). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fourth Informational Supplement. CLSI Document M100-S24 34, 1–214.

De Medici, D., Anniballi, F., Wyatt, G. M., Lindström, M., Messeilhausser, U., Aldus, C. F., Delibato, E., Korkeala, H., Peck, M. W. & others authors (2009). Multiplex PCR for detection of botulinum neurotoxin-producing clostridia in clinical, food, and environmental samples. Appl Environ Microbiol 75, 6457–6461.
Fox, J. G., Ge, Z., Whary, M. T., Erdman, S. E. & Horwitz, B. H. (2011). Helicobacter hepaticus infection in mice: models for understanding lower bowel inflammation and cancer. *Mucosal Immunol* 4, 22–30.

Hall, J. D., McCroskey, L. M., Pincomb, B. J. & Hatheway, C. L. (1985). Isolation of an organism resembling Clostridium barati which produces type F botulinum toxin from an infant with botulism. *J Clin Microbiol* 21, 654–655.

Hannett, G. E., Schaffzin, J. K., Davis, S. W., Fage, M. P., Schoonmaker-Bopp, D., Dumas, N. B., Musser, K. A. & Egan, C. (2014). Two cases of adult botulism caused by botulinum neurotoxin producing *Clostridium baratii*. *Anaerobe* 30C, 178–180.

Huang, W. C., Lee, W. S., Chang, T., Ou, T. Y. & Lam, C. (2012). Emphysematous cholecystitis complicating liver abscess due to *Clostridium baratii* infection. *J Microbiol Infectol* 45, 390–392.

Iaria, C., Stassi, G., Salpietro, D. C., La Mazza, A., Silipigni, L., Arena, A., Costa, G. B. & Cascio, A. (2007). *Clostridium baratii* bacteremia associated with Kawasaki syndrome. First case report. *New Microbiol* 30, 481–484.

Krieg, N. R., Ludwig, W., Whitman, W. B., Hedlund, B. P., Paster, B. J., Staley, J. T., Ward, N. & Brown, D. (2010). *Bergey’s Manual of Systematic Bacteriology*, 2nd edn, vol. 4. New York, NY: Springer-Verlag.

Ngo, J. T., Parkins, M. D., Gregson, D. B., Pitout, J. D., Ross, T., Church, D. L. & Laupland, K. B. (2013). Population-based assessment of the incidence, risk factors, and outcomes of anaerobic bloodstream infections. *Infection* 41, 41–48.

Park, Y., Choi, J. Y., Yong, D., Lee, K. & Kim, J. M. (2009). Clinical features and prognostic factors of anaerobic infections: a 7-year retrospective study. *Korean J Intern Med* 24, 13–18.

Sebald, M. & Petit, J. C. (1997). *Laboratory Methods Anaerobic Bacteria and Their Identification*, 2nd edn, pp. 189–197. Paris: Institute Pasteur.

Shu, C. C., Yao, M., Hung, C. C., Ku, S. C., Yu, C. J. & Chang, Y. L. (2008). Lung abscess due to *Clostridium baratii* infection in a patient with invasive pulmonary aspergillosis. *J Clin Microbiol* 46, 1153–1154.

Somier, J. (2002). *Wadsworth Anaerobes Bacteriology Manual*, 6 edn, Edited by H. R Josiemies-Somier, P Summanen, D. M Citron, E. J Baron, H. M. Wexler & F. M. Finegold. Belmont, CA, USA: Star Publishing Company.

Trehard, H., Poujol, I., Mazuet, C., Blanc, Q., Gillet, Y., Rossignol, F., Popoff, M. R. & Jourdan Da Silva, N. (2016). A cluster of three cases of botulism due to *Clostridium baratii* type F, France, August 2015. *Euro Surveill* 21, 4.

Tsybuliak, G. N. & Epifanov, M. V. (2009). [Clostridial forms of anaerobic infections of wounds]. *Vestn Khir Im I I Grek* 168, 111–115 (In Russian).