Fatal Bacteremia Due to *Anaerobiospirillum succiniciproducens*: First Description in Brazil

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Anaerobiospirillum succiniciproducens **is an anaerobic, Gram-negative, spiral shaped bacteria, which is motile by means of bipolar tuffs of flagella. This organism appears to be a rare cause of bacteremia in humans, and it usually affects patients submitted to immunosuppressive therapy.** ***Anaerobiospirillum succiniciproducens*** **resembles** ***Campylobacter*** **spp. in Gram-stained preparations, however, it is considered resistant to most antimicrobial drugs that are used to treat** ***Campylobacter*** **infections. We observed Gram-negative, spiral shaped bacteria in Gram-stained preparations from blood culture flasks. Growth occurred only under anaerobic incubation, and identification to the species level was achieved by PCR amplification of the 16S rRNA gene, followed by direct sequencing and a GenBank homology search. To the best of our knowledge, this is the first reported Brazilian case of** ***Anaerobiospirillum succiniciproducens*** **bacteremia.**

Key Words: Bacteremia; ***Anaerobiospirillum*** **spp.;** ***Anaerobiospirillum succiniciproducens*****.**

Spiral-shaped bacteria have been observed in the gastrointestinal tract of humans as well as in animals, and their role as the cause of diseases has been studied since then [1]. Davis et al. [2] isolated spiral-shaped bacteria from the throat and feces of a Beagle dog, and described the genus *Anaerobiospirillum* for the first time in 1976. *Anaerobiospirillum spp*. comprises a group of spiral shaped Gram-negative anaerobic rods; they are motile by bipolar tufts of flagella, and are frequently isolated from feces of cats and dogs [3,4].

The first report of bacteremia caused by *Anaerobiospirillum succiniciproducens* was described in 1981 by Rifkin and Opdyke [1]. McNeil et al. [5], reviewed all cases of bacteremia caused by anaerobic Gram-negative bacilli in the United States, occurring between 1975 and 1986. They identified 22 cases of bacteremia due to *A. succiniciproducens* and observed that most of these patients had some degree of immunosuppression. In 1990, using Skirrow *Campylobacter* medium incubated at 40°C to 43°C for 48 to 72 hours under a reduced oxygen atmosphere, Malnick et al. [4] isolated *A. succiniciproducens* from the feces of 18 patients in the United Kingdom. Malnick et al. [6] also described a new selective medium specific for *Anerobiospirillum* spp., which is incubated at 37°C for 48 hours under anaerobic conditions.

Anaerobiospirillum succiniciproducens is known as a rare cause of diarrhea and bacteremia in humans. Initial infection is usually associated with the gastrointestinal tract, and this could be the primary portal of entry for this organism [7,8]. Moreover, bacteremia is usually preceded or accompanied by gastrointestinal symptoms [7,9]. This organism is reported to be susceptible to carbenicilin, chloramphenicol, and cephalothin, and resistant to vancomycin and nalidixic acid. Variable degrees of susceptibility have been reported against penicillin, ampicillin, erythromycin, clindamycin, and metronidazol [3]. Susceptibility to

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cefuroxime, amoxacillin-clavulanic acid, cefoxitin and imipenem is described by other authors [7,8].

Anaerobiospirillum spp. may be mistakenly identified as *Campylobacter* spp. due to its similar shape on Gram staining and positive motility. Differentiation between these two species, however, can be accomplished by demonstration of tufts of polar flagella by electronic microscopy [1,3,10]. Useful biochemical markers for *Anaerobiospirillum* spp. include: negative catalase, oxidase, indol, and nitrate reduction [3]. Additional tests using API-ZYM (bio-Merieux) will reveal positive reactions for: leucine arylamidase, phosphohydrolase, α-glicosidase and N-acetyl- β-glucosaminidase, and a negative reaction with β-galactosidase. Carbohydrate fermentation is positive for fructose, glucose, maltose and sucrose, and negative for lactose and raffinose [8,11]. Determination of the 16S rRNA gene by PCR amplification and sequencing, however, is the most accurate method for identification and classification of *Anaerobiospirillum* spp. [3].

Case Report

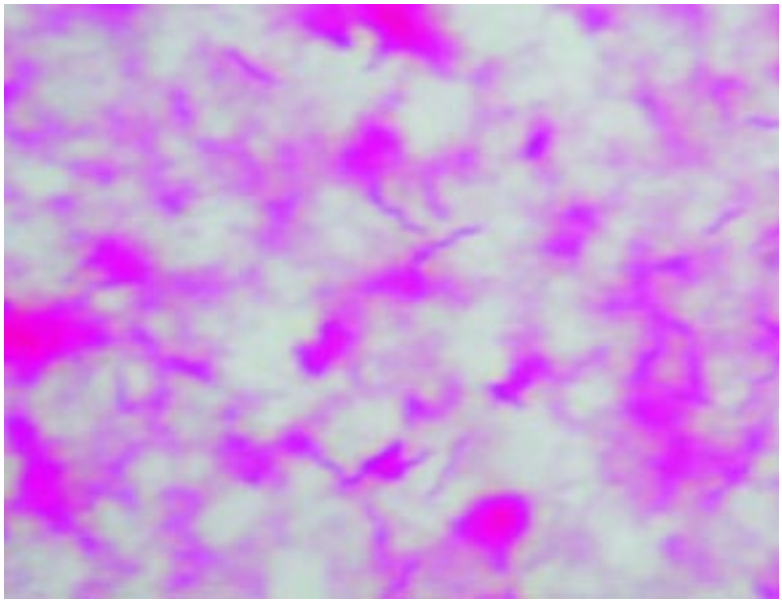
A 73 year-old female patient was admitted to the Emergency Room of the Grupo Hospitalar Conceição (GHC), Porto Alegre, RS, Brazil, in May, 2003, with right hemiparesy and transitory dysphasia. Her previous medical records revealed cigarette smoking, diabetes mellitus type II, arterial hypertension, impaired cardiac function, and a cerebrovascular accident with ischemia in the past 30 days associated with paresthesia of the left upper limb. The patient developed fever (38.4°C) and clinical signs of infection six days after her admission to the hospital. Hematological tests revealed that her total white blood cell count was 10,610/mm^3 (13% bands, 74.8% neutrophils). *Staphylococcus aureus* was isolated from blood cell culture, and oxacillin plus gentamycin were administered based on the susceptibility test results for this organism. The patient then complained of abdominal pain, nausea, and vomiting.

From the 8th day after her admission, she presented with clinical signs of sepsis, which was evidenced by hypotension. Fever (37.8ºC) persisted during the next week, together with clinical signs of renal and pulmonary involvement. *Staphylococcus aureus* was isolated again from a new blood culture, and the therapy was changed to vancomycin, after consulting with the hospital infection control team. Nevertheless, the patient continued to have fever, nausea, vomiting, and she also presented diarrhea.

On day 19, the therapeutic regimen was changed again, and oxacillin was given as a substitute for vancomycin, since oxacillin-sensitive *S. aureus* was isolated from her urine culture. On day 24, there was an aggravation of her sepsis condition, followed by marked metabolic acidosis, renal insufficiency, chest pain (angina), clinical evidence of pulmonary edema, and subsequent cardiorespiratory arrest. Blood counts revealed 16,600 white blood cells per mm^3 (1% myelocytes, 2% metamyelocytes, 28% bands). The patient was transferred to the intensive care unit (ICU) following general worsening of her clinical conditions, including septic shock and coma. Empirical piperacillin-tazobactam therapy was initiated and a new set of blood cultures for aerobic and anaerobic organisms was ordered.

The new set of blood cultures were positive and the hospital clinical microbiology laboratory noted the presence of spiral shaped, *Campylobacter*-like, Gram-negative rods in all positive flasks (anaerobic and aerobic). The blood culture bottles were sent to the Bacteriology & Molecular Biology section of Laboratório Weinmann Ltd., where *Anaerobiospirillum succiniciproducens* was identified. The clinical conditions of the patient deteriorated and she died on day 27 post admittance. The spiral shaped Gram-negative rods from one of the blood culture bottles are shown in Figure 1. The following agar plates were streaked in an attempt to isolate this organism: sheep blood agar (bioMérieux), incubated at 35°C for 48 h under increased CO_2 atmosphere, and Schaedler agar (bioMérieux) incubated at 35°C under anaerobic conditions for 48 h. Campylobacter agar (Oxoid) was also used and incubated at 42°C for 48 h under a microaerophilic atmosphere. Bacterial growth was observed only on

Figure 1. The spiral shaped Gram-negative anaerobic rods of *Anaerobiospirillum succiniciproducens*.



Schaedler agar. Colonies were translucid, circular, convex, and non-hemolytic. Gram staining from a colony suspension revealed the same spiral shaped Gram-negative bacteria observed in the original blood culture bottles.

Correct identification to the species level was accomplished by PCR amplification of the 16S rRNA gene (ca. 1,500 bp), microaerophilic, amplified using primers 285 and 261 [12], and the first 500 bases determined by sequencing using primer 16S (5' – TATTACCGCRGCTGCTGG – 3'), as described below, followed by direct DNA sequencing. PCR products obtained with different primer sets were cleaned with shrimp alkaline phosphatase and Exonuclease I (USB), and submitted to direct sequencing using the BigDye Terminator kit (Applied Biosystems), as directed by the manufacturer's instructions.

Discussion

Anaerobiospirillum spp. is an uncommon cause of gastroenteritis and bacteremia, as indicated by previous studies [3]. Most of the patients presenting with diarrhea caused by this organism had no other basal disease and recovered from the infection. On the other hand, patients with bacteremia due to this organism often have a clinical record of previous diseases, such as alcoholism, malignancies, immunosuppression, diabetes mellitus, other gastrointestinal disorders, atherosclerosis, and dental caries [3,5].

In our case, the patient had diabetes mellitus type II, among other symptoms, and stayed for a long period in the hospital, where she was receiving extended-spectrum antibiotics, exacerbating her immunodepression. Under these conditions, patients are known to be more susceptible to infections by *Anaerobiospirillum* spp. [5]. Unfortunately the patient died before any results from the anaerobic culture and identification could be used to change the antibiotic treatment to cover this bacterium. Despite her gastrointestinal symptoms, no fecal sample was submitted for culture. Ordering of fecal cultures for inpatients staying for long periods in the hospital is neither common nor considered adequate [13]. Any attempt to isolate uncommon pathogens from fecal samples should be discussed with the

microbiology specialists, since, as in this case, these organisms usually will not grow on media commonly used to screen for specific enteropathogenic organisms.

Interestingly, *S. aureus* was isolated from the blood culture bottles on more than one occasion, and though appropriate therapy was given, as judged by the results of the susceptibility tests, the patient's general condition rapidly deteriorated. We hypothesize that *A. succiniciproducens* was already present together with the *S. aureus,* however, it was not initially detected due to the concomitant presence of the latter, which is easily seen in Gram-stained preparations. Moreover, *S. aureus* was easily isolated from subcultures, whilst *A. succiniciproducens* only grew on special media under anaerobic atmosphere. *Anaerobiospirillum succiniciproducens* is also resistant to vancomycin, which was used to treat *S. aureus* bacteremia in this patient.

Rapid identification of this uncommon anaerobic microorganism is important to guide antimicrobial therapy, since it is known to be naturally resistant to metronidazol [7], one of the first-line drugs used to treat anaerobic infections.

The number of cases involving *A. succiniciproducens* is increasing worldwide, being reported from countries, such as the USA, South Africa, China (Hong Kong), Germany, New Zealand, Australia, the UK, Spain and Israel [3,8]. To the best of our knowledge, this is the first case of bacteremia due to *A. succiniciproducens* reported in Brazil. The increased awareness of the importance of these unusual agents as the cause of human diseases, and the use of improved methodologies by clinical microbiology laboratories is crucial for the rapid isolation and identification of these organisms.

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