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Identification of *Anaerobiospirillum succiniciproducens* by MALDI-TOF mass spectrometer. A bacteremia in an immunocompetent patient

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Sir,

A 55-year-old man with a history of distal esophageal adenocarcinoma, treated with a minimally invasive esophagectomy, and a recently implanted DDD pacemaker due to persistent vasovagal syncope presented to the emergency department with 72 hour-long febrile syndrome associated with mild odynophagia and dry cough. These symptoms were relatively controlled with the administration of paracetamol. In the complementary tests no alarming data or signs were observed, except a C-reactive protein of 29 mg/L, as well as a mild leukocytosis of 12,000 μL^{-1} with an 85% of neutrophils in the blood tests. Blood culture was obtained (2 extractions) and the patient was discharged home with antipyretic treatment.

The two anaerobic flasks of the blood culture were positive at 66 h and 67 h of incubation, with Gram stain showing curved Gram-negative rods. Given the morphology of the bacilli in the Gram stain, the sample was inoculated on BDM Campy BAP, Brucella and MacConkey agars. Direct identification of the blood culture was conducted by MALDI-TOF mass spectrometer (Bruker, Massachusetts, USA) following the procedure proposed by Urrutikoetxea-Gutiérrez M *et al.* and *Anaerobiospirillum* spp. was obtained as identification with a value between 1.7 and 2.0 [1]. After the notification of this result the patient was admitted for completing the study and treated with intravenous imipenem (500 mg/6 h).

During the previous days he reported having presented inflammation, erythema and pain in the 4th finger of the left hand compatible with cellulitis. In addition, the patient stated that since the beginning of the year he has adopted a pet dog that has scratched him on one occasion and has had some episodes of diarrhea in the previous weeks.

After 48 h of incubation of the plates on anaerobic condi-

tions, growth was observed only on Brucella agar as flat translucent colonies with negative catalase and oxidase reactions, confirming the identification of *A. succiniciproducens* by MALDI-TOF MS with a value of 2,10. Identification was confirmed by 16S rRNA sequencing with *A. succiniciproducens* as result with an homology percentage of 88,3%. Antibiotic susceptibility was performed by E-test on Brucella agar in anaerobiosis at 37°C during 48h of incubation. Following the EUCAST (version 8.1, 2018) anaerobic Gram-negative rods and PK-PD cut-off points, the strain was susceptible to amoxicillin/clavulanate, cefuroxime, cefotaxime, imipenem, ertapenem, moxifloxacin and levofloxacin but resistant to penicillin, amoxicillin, clindamycin and metronidazole (Table 1).

A thoracoabdominal CT scan was performed, which ruled out infectious focus at those levels, as well as a transthoracic echocardiogram, which did not show data suggestive of endocarditis. After 72 h of admission, the patient evolved favorably and was discharged with 875 mg/125 mg of oral amoxicillin/clavulanate. However, due to an allergic reaction the antibiotic treatment had to be replaced to 400 mg/24 h of moxifloxacin, fulfilling 10 days of oral antibiotic treatment with good evolution.

The genus *Anaerobiospirillum* consists of only two species, *A. succiniciproducens* and *Anaerobiospirillum thomasi*, which were both first isolated in 1967 from throat and animal stool samples [2]. *Anaerobiospirillum* spp. can be differentiated from other similar microorganisms such as *Campylobacter* spp. using classical microbiological techniques, being negative to the catalase and oxidase reactions and having an obligate anaerobic growth. Moreover, there are other structural differences such as lophotriche flagellation, an electron-dense ring located below the flagellated pole, or the existence of fibrillar structures that are oriented along the longitudinal axis that appear to be unique characteristics of this microorganism [4].

They are Gram-negative curved rods, which are part of the gastrointestinal commensal flora of cats and dogs and

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Table 1 Susceptibility of the *A. succiniciproducens* isolate

Antibiotic	MIC (mg/L)	Interpretation
Penicillin	> 32	Resistant
Amoxicillin	16	Resistant
Amoxicillin/clavulanate	0.5	Susceptible
Cefuroxime	0.5	Susceptible
Cefotaxime	0.064	Susceptible
Imipenem	0.12	Susceptible
Ertapenem	0.012	Susceptible
Clindamycin	>32	Resistant
Metronidazole	>256	Resistant
Moxifloxacin	0.25	Susceptible
Levofloxacin	0.5	Susceptible

therefore having contact with animal faeces is probably one of the routes of transmission of infection in humans [3]. Another possible route of transmission could be the bite of colonized animals, as an *Anaerobiospirillum* bacteremia after a cat bite has been previously described [5].

Around 50-60 human infections caused by *A. succiniciproducens* have been described, being bacteremia or gastro-intestinal infections the most frequent cases [6,7]. It has also been described as a cause of knee prosthesis infection [8]. Most infections occur in patients with comorbidities, ethylism or immunosuppression, however, as in the presented case it can also cause infection in immunocompetent patients [6-10]. Although the incidence of these infections is low, mortality is around 25-30% of cases, therefore, rapid action is essential [6,8].

For all these reasons, identification by MALDI-TOF mass spectrometry is a very important diagnostic tool, since it is a fast and accurate method that has been previously tested for the identification of *A. succiniciproducens* [6,7]. In addition, direct identification of the blood culture by MALDI-TOF makes the process even faster, being useful in the identification of potentially fatal and growth-demanding organisms such as *Capnocytophaga canimorsus* [11]. While 16S ribosomal RNA gene sequencing is a more accurate method of identification, it is also more complicated, laborious and takes longer to arrive at identification. In fact, since Fox B *et al.* conducted their study in 2018 the Bruker MALDI-TOF database has been updated and includes now the main spectra of *A. succiniciproducens* [7].

The most appropriate antibiotic treatment of infections caused by *A. succiniciproducens* remains to be determined due to limited experience. In the previous cases reported, this microorganism was sensitive to beta-lactams associated with beta-lactamase inhibitors, cephalosporins or chloramphenicol,

showing variable sensitivity to penicillin and ampicillin [8]. It is usually resistant to anaerobic antibiotics such as metronidazole and clindamycin, as in our case. There are no specific fluoroquinolone cut-off points for anaerobic microorganisms, however, *A. succiniciproducens* showed in vitro sensitivity to gemifloxacin and trovafloxacin in the article by Goldstein EJ *et al.* and, furthermore, in the manuscript by Kelesidis T, the patient was treated with satisfactory result with levofloxacin [12,13]. In our case, following EUCAST PK/PD cut-off points, both levofloxacin and moxifloxacin showed in vitro sensitivity. In fact, the patient received 10 days of oral treatment with moxifloxacin as sequential therapy after 72 h of intravenous treatment with imipenem without the appearance of complications, which adds further evidence to the possible in vivo activity of this microorganism.

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CONFLICT OF INTEREST

Authors declare have no conflict of interest

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