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Letter to the Editor

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Identification of curved Gram-negative rods by MALDI-TOF mass spectrometer in a patient with Fournier´s gangrene. A bacteremia caused by *Desulfovibrio desulfuricans* and *Escherichia coli*

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Sir,

Desulfovibrio desulfuricans are obligate anaerobic gram-negative rods, curved, sulphate-reducing and commensals of the gut microbiota. *Desulfovibrio* genus comprises more than 60 species, however, only *D. desulfuricans, Desulfovibrio fairfieldensis, Desulfovibrio vulgaris, Desulfovibrio piger, Desulfovibrio legallii* and *Desulfovibrio intestinalis* have been clinically isolated up to now [1].

A 69-year-old patient with a history of untreated chronic obstructive pulmonary disease was found at home with deterioration of consciousness, respiratory failure and poor general condition. Physical examination showed signs of hemodynamic instability with blood pressure of 63/33 mmHg, oxygen saturation of 78%, poor perfusion, abdominal lividity, capillary refill > 2 seconds, sweaty. Furthermore, the patient presented extensive gangrene of the scrotal area, penile base and perianal region, crepitant on palpation and incarcerated left inguinal hernia. He was taken to the emergency department with a diagnosis of Fournier's gangrene, with the need for vasoactive drugs. Initial laboratory tests showed: hemoglobin 8.9 g/dL, 29750 leukocytes with 91.50% neutrophils, glucose 98 mg/dL, urea 229 mg/dL, GPT 139 U/L, creatinine 2.81 mg/dL, glomerular filtration rate 22 mL/min/1. 73 m2, creatine kinase 6362 U/L, C-reactive protein 329.46 mg/L, prothrombin index 41%, INR 1.9, activated partial thromboplastin time 38 seconds, fibrinogen 634 mg/dL. A blood culture sample was obtained, he underwent surgery with debridement (intraoperative sample was also obtained) and was admitted to the resuscitation department with 2 g/8 h of IV meropenem, 600 mg/8 h of IV clindamycin and 500 mg/24 h of IV daptomycin.

The blood culture was incubated in the BD BACTEC® system (Becton Dickinson, New Jersey, USA) and one of the aerobic vials (BD BACTEC™ Plus Aerobic/F Culture Vials) was positive at 14 h of incubation. Gram staining showed Gram-negative bacilli and it was subcultured on chocolate (incubated at 37 °C in 7% CO_2 atmosphere) and MacConkey (incubated at 37 °C in aerobiosis) agars. After 24 h, growth was identified as *Escherichia coli* by MALDI-TOF mass spectrometer (Bruker, Massachusetts, USA) with a score of 2, 33. One of the anaerobic vials (BD BACTEC™ Lytic/10 Anaerobic/F Culture Vials) was positive after 4 days of incubation, whose Gram staining revealed curved, short, spiral Gram-negative rods (Figure 1, right image). It was subcultured on chocolate and BD® Brucella Blood with Hemin and Vitamin K1 agars (incubated at 37°C in an anaerobic atmosphere) and after 72 h, small colonies 0.5-1 mm in diameter, round and shiny were observed on Brucella agar (Figure 1, left image) and identified as *D. desulfuricans* by MALDI-TOF MS (Bruker, Massachusetts, USA) with a score 2.2. The antibiotic susceptibility of *D. desulfuricans* was studied by MICs with antibiotic gradient strips or E-test® (Liofilchem, Teramo, Italy) on Brucella agar under anaerobic conditions. The strain was susceptible according to EUCAST 2020 breakpoints guidelines (Gram-negative anaerobes) to amoxicillin + clavulanic acid (MIC=0.12 mg/L), imipenem (MIC=0.25 mg/L), meropenem (MIC= 0.06 mg/L), clindamycin (MIC=0.5 mg/L), metronidazole (MIC=0.06 mg/L) and resistant to piperacillin/tazobactan (MIC 128 mg/L), although currently *D. desulfuricans* does not have cut-off breakpoints.

The intraoperative sample was inoculated on chocolate, CNA and TSA agars with 5% sheep blood (Becton Dickinson, New Jersey, USA), MacConkey, Brucella y BBE with Amikacin agars. Chocolate, TSA with 5% of sheep blood and CNA agars were incubated in microaerophlic conditions, MacConkey agar in aerobiosis, whereas Brucella and BBE with Amikacin agars in anaerobiosis. Gram staining showed no leucocytes and no microorganisms, nevertheless *E. coli* and *Bacteroides thetaiotaomicron* grew at 24 h in McConkey agar and at 48h in Brucella agar by MALDI-TOF MS, respectively. *D. desulfuricans* did not grew in these subcultures, but its presence cannot be ruled

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Figure 1 **Left figure: subculture of the positive anaerobic bottle in Brucella agar after 72-96 h of incubation in anaerobic conditions. Brightly and small colonies growing at the bottom of the agar were identified as *Desulfovibrio desulfuricans* with MALDI-TOF MS.**
Right figure: gram staining of the anaerobic bottle showed curved Gram-negative rods (scale x1000).

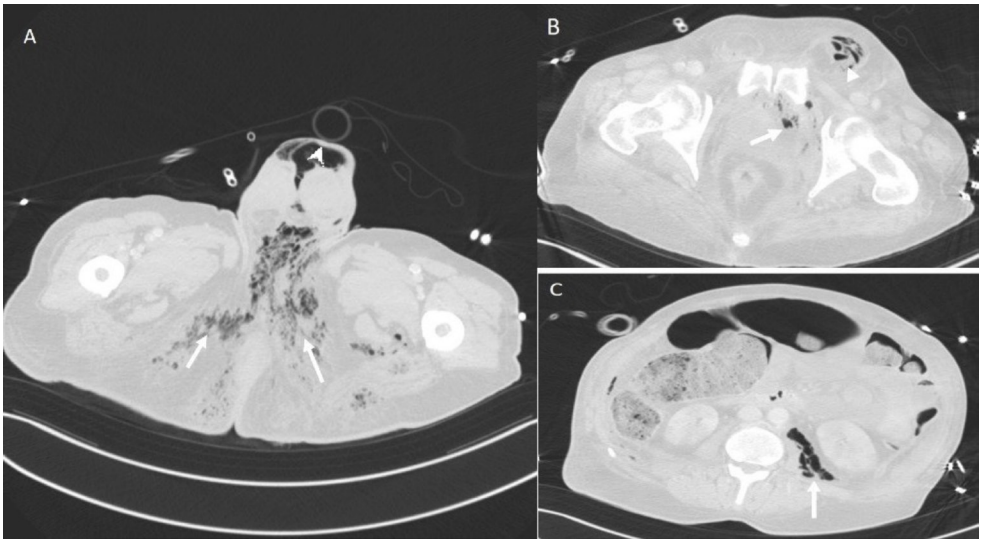

Figure 2 **2A: abdominopelvic CT with lung-window showing a large amount of gas dissecting the fatty and muscular planes of the gluteal and perineal region (arrows) with extension to the scrotum (arrowhead). In figure 2B, we observe ascension towards the obturator internus muscle (arrow) and inguinal canal (arrowhead). In figure 2C, we observe how the emphysema extends into the retroperitoneal space, dissecting mainly the left posterior pararenal fascia (arrow).**

out given its fastidious growth (the subcultures were discarded after the identification of these two microorganisms).

A thoracic-abdominal-pelvic CT scan showed findings consistent with Fournier´s gangrene, as well as neoplastic disease in the rectum with possible fistulization towards the ischiorectal collection and liver metastases (Figure 2). Several hours after surgery, the patient presented abruptly increased hemodynamic instability with multiorgan failure. Subsequently, he went into cardiorespiratory arrest without improving after cardiopulmonary resuscitation maneuvers and finally died.

D. desulfuricans is characterized by the presence of a pigment, desulfoviridin (with blue-green fluorescence at acid pH and red fluorescence at alkaline pH) [2]. *D. desulfurincans* has rarely been described in human infections; however, it has been previously isolated causing bacteremia [3], liver abscesses [4] or septic arthritis [5]. Immunosuppression (malignancy or

diabetes), gastrointestinal disease, history of trauma or previous surgery are known risk factors for these infections [4,5]. Our patient did not have medical history of interest, but the CT-scan performed during hospitalization showed possible premalignancy

The identification of fastidious microorganisms such as *D. desulfuricans* has been hindered in the past years by the limitations of biochemical methods or by the limited availability of molecular identification technics such as 16S rRNA gene sequencing in most laboratories. However, in recent years, this has changed with the introduction of the MALDI-TOF mass spectrometer into the routine of many laboratories. It is an accurate, inexpensive and accessible tool relative to molecular methods, which has shortened the time to identify these bacteria [6].

Optimal treatment of *D. desulfuricans* infections is still unclear; however, it should be taken into account that this anaerobe may produces beta-lactamases [7]. Metronidazole, clindamycin, chloramphenicol or carbapenems can be used, whereas amoxicillin/clavulanic usually shows good in-vitro activity and most strains present high MICs to piperacillin/tazobactam [8] (as in our case). Given the general susceptibility of the strains studied so far, piperacillin/tazobactam and cephalosporins should be avoided as antibiotic treatment. However, given the good evolution in most cases [1,3-6,8], combined treatments do not seem necessary (except in infections associated with other gram-negative or gram-positive microorganisms, as well as in the empirical treatment of severe intra-abdominal infections). Despite correct antibiotic treatment, control of the focus is mandatory in most infections like the presented case [9].

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None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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