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Letter to the Editor

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A case report of multiple abscess co-infected with *Eggerthella lenta* and *Desulfovibrio desulfuricans* identificated with MALDI-TOF mass spectrometer

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Sir,

In this article, we report a case of multiple abscesses in a patient with history of malignancies and a recent resective colon surgery caused by two nutritionally demanding anaerobic microorganisms. *E. lenta* is an anaerobic non-spore- forming gram-positive bacilli belonging to the *Eggerthellaceae* family, which includes several bacteria commonly found in the gut microbiota [1]. *D. desulfuricans* are obligate anaerobic, curved, motile, sulphate-reducing, gram-negative bacilli and commensals of the gut microbiota, belonging to the *Desulfovibrionaceae* family. This genus comprises more than 60 species, however, only 6 have been isolated from human infections: *Desulfovibrio desulfuricans, Desulfovibrio fairfieldensis, Desulfovibrio vulgaris, Desulfovibrio piger, Desulfovibrio legallii* and *Desulfovibrio intestinalis* [2]. They are characterized by the presence of a pigment, desulfoviridin, which fluoresces red at alkaline pH and blue-green at acidic pH under long-wavelength ultraviolet light [3].

An 80-year-old woman with an ileostomy came to the emergency department with expulsion of blood-purulent contents through an infraumbilical midline incision from a recent colectomy surgery. Her medical history included an infiltrating ductal carcinoma of the breast 9 years earlier treated by quadrantectomy, axillary emptying and a combination of radiotherapy, chemotherapy and letrozole. Furthermore, she undergone an open colectomy with resection up to the transverse colon, splenic angle and the descending colon preserving the sigma due to complicated bowel obstruction one year ago. The patient also suffered some years ago from a moderately differentiated intestinal adenocarcinoma and poorly cohesive gastric carcinoma, being treated by total gastrectomy, chemotherapy and radiotherapy. At the physical examination, the patient was hypotense (85/52 mmHg), eucardic (69 bpm) and afebrile (36.3ºC). A blood test showed an hemoglobin of 11.9 g/dL, 450000 platelets (150,000-400,000), a hematocrit of 36%, normal renal and liver function, INR (International normalized ratio) of 1.3 and elevated acute phase reactants with C-reactive protein of 178 mg/L, 17000 leukocytes (4500-11000) with 91% neutrophils and fibrinogen of 856 mg/dL. A computed tomography (CT) scan showed an overinfected amphractuous pelvic collection (Figure 1A and B), extending from the intraperitoneal space in Douglas' *cul-de-sac* to the anterior extraperitoneal space where there was cutaneous fistulization, as well as an independent collection at the level of the left perisigmoid. The patient was admitted for collection drainage by interventional ultrasound, placing an anterior drainage catheter and aspirating 20 mL of purulent material. However, the collection was not completely drained and a second drainage tube was placed through the right posterior approach, aspirating another 20 ml. Two peritoneal pig-tail tub drainages, 12 Fr in diameter and 25 cm in length, were used for evacuating purulent contents of abscesses.

A sample was sent to the laboratory for microbiological analysis and two sets of blood culture bottles were obtained. Finally, 250000 U of urokinase was administered and 4 g/0.5 g each 8 hours of IV piperacillin/tazobactam was prescribed. The sample was inoculated in Thioglycollate^{R} enrichment broth, chocolate agar, Columbia CNA agar, tryptic soy agar (TSA) with 5% of sheep blood (Becton Dickinson, New Jersey, USA), MacConkey agar, Brucella agar with Hemin and Vitamin K1 and Bacteroides bile esculin (BBE) agar with amikacin. Thioglycolate^{R} enrichment broth, chocolate agar, CNA agar and TSA with 5% of sheep blood were incubated at 37°C under aerobic conditions with 7.5% CO_2 for 96 h, McConeky agar at 37°C under aerobic conditions for 96 h, while Brucella and BBE agars were incubated at 37°C under anaerobic conditions for 72 h. Blood cultures bottles were incubated in the BD^{R} BACTEC FX (Becton Dickinson, New Jersey, USA), being negative after five days of incubation.

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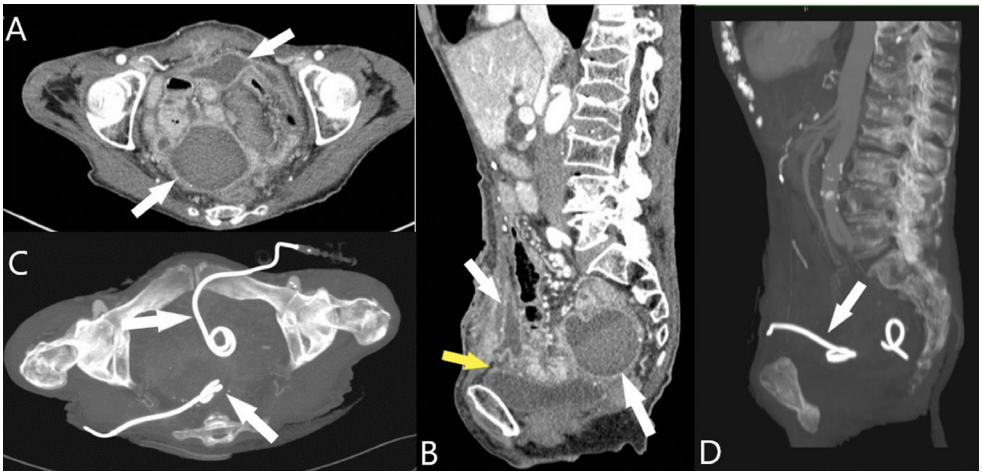

Figure 1 Abdominopelvic CT after contrast, axial image (A) and sagittal reconstruction (B) where we observe pelvic collections/abscesses (white arrows) with fistulization towards the straight abdominal musculature (yellow arrow). Axial (C) and sagittal (D) MIP reconstructions after placement of pig-tail catheters for drainage of the collections (white arrows).

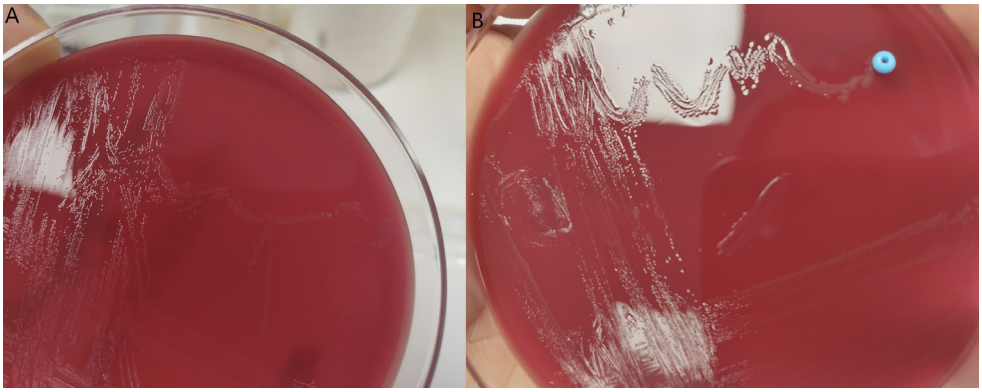

Figure 2 A: growth of brightly, transparent and tiny colonies in Brucella agar identified as *Desulfovibrio desulfurican* with MALDI-TOF MS. B: growth of yellowish and transparent colonies identified as *Eggerthella lenta* with MALDI-TOF MS.

Gram staining of the sample showed 10-25 leukocytes/field and no microorganisms. Two different microorganisms grew on Brucella agar after 72 h (Figure 2), being identified by MALDI-TOF mass spectrometer (Bruker, Massachusetts, USA) as *Eggerthella lenta* and *Desulfovibrio desulfuricans* (Figure 2) with values of 2.16 and 2.33, respectively (MALDI-TOF Biotyper^{R}, MBT IVD, Library 9.0). The remaining culture media did not show growth. A Gram staining was performed directly from the colonies of *D. desulfuricans* in order to visualize its characteristic curved morphology. The antibiotic susceptibility test was performed by gradient strips or MIC Test Strip^{R} (Liofilchem, Teramo, Italy) on Brucella agar. Following CLSI breakpoints (M100, Performance Standards for Antimicrobial Susceptibility Testing, 2022), *E. lenta* was susceptible to amoxicillin/clavulanic acid (MIC = 0.064 mg/L), clindamycin (MIC= 0.016 mg/L) and metronidazole (MIC= 0.125 mg/L), while showing resistance to piperacillin/tazobactam (CMI = 32/4 mg/L). *D. desulfuricans* showed low MICs to amoxicillin/clavulanic acid (MIC = 0.125 mg/L), clindamycin (MIC= 0.064 mg/L) and metronidazole (MIC= 0.125 mg/L), being resistant to piperacillin/tazobactam (MIC > 256 mg/L). According to CLSI recommendations, for both susceptibility study methods (agar dilution and broth microdilution), Brucella agar and Brucella broth must be used and supplemented with Hemin, vitamin K1 and laked sheep

blood, being incubated at 36ºC ± 1ºC under anaerobic conditions for 46-48 h (broth microdilution) or 42-48 h (agar dilution). Antibiotic treatment was changed to 2 g/200 mg/8 h of IV amoxicillin/clavulanic acid.

Five days later, a control CT scan was performed (Figures 1C and 1D) showing a reduction of the pelvic collection. A small amount of fluid evacuated through the drainage tubes was observed some days after, in which both *E. lenta* and *D. desulfuricans* were identified again but with poor growth. The following blood tests showed a decrease in acute phase reactants and the patient improved its condition. She was finally discharged after 13 days of antibiotics with close observation of the surgical drains in the following two weeks with no treatment. The patient expired one month later after sudden worsening of the general condition.

The spectrum of possible infections (both monomicrobial and polymicrobial) that can be caused by these two growth fastidious microorganisms is yet to be determined due to scarce case reports. The review of Wang J et al. describes how *E. lenta* can cause local infections as abscesses or systemic infections such as bacteremia due to bacterial translocation [4]. More specifically, it has been associated with infections such as liver abscesses [4], pyomyositis [5] or bacteremia [6] in the context of multiple abscesses [7], among others. *D. desulfuricans* has rarely been described in human infections, however, it has been isolated causing bacteremia [8,9], liver abscesses [10] or septic arthritis [11]. The risk factors found that predispose to infections by these microorganisms are those related to immunosuppression (malignancy or diabetes), gastrointestinal disease, as well as a history of trauma or previous surgery [5,8,11].

Since the growth of these microorganisms is slow and fastidious, their identification in the past has been hindered by the limitations of biochemical methods or by the scarce availability of molecular identification methods such as 16S rRNA gene sequencing in most laboratories. *D. desulfuricans* may be identified by growth after sodium formate/sodium fumarate stimulation, the production of different biochemical reactions such as nitrate, urea, desulfoviridin or H_2S ("Sulfur, Indole, Motility" or SIM culture) or its mobility [2]. In the other hand, *E. lenta* does not ferment carbohydrates (the cells do not hydrolyze esculin, hippurate and gelatin), but it produces ammonia from arginine, and H_2O_2 from agar medium containing 1% arginine [12]. Furthermore, *E. lenta* can produce H_2S in a triple sugar iron agar, but cannot produce it in SIM culture medium. However, in recent years this has changed with the introduction of the MALDI-TOF mass spectrometry into the routine of many laboratories. This accurate, inexpensive and accessible tool has shortened the time needed to identify these bacteria compared to molecular methods [5, 13].

Regarding the susceptibility of *E. lenta*, Bo J et al. [14] reviewed the antibiotic susceptibility data of isolates between 2010 and 2020. *E. lenta* strains seem to be susceptible to metronidazole, amoxicillin-clavulanate, carbapenems, vancomycin and clindamycin, with high MICs in piperacillin/tazobactam and moxifloxacin in some strains. The optimal treatment has not yet been established in *D. desulfuricans*; however, it should be consider the possibility that this bacteria may produce beta-lactamases [15]. It could be treated with metronidazole, clindamycin, chloramphenicol or carbapenems, with variable MICs to piperacillin/tazobactam [8]. In our case, both microorganisms showed low in-vitro activity to piperacillin-tazobactam (empirical antibiotic treatment prescribed in this case, then changed to amoxicillin/clavulanic acid), but he patient improved its condition due to a proper drainage of the collections, which emphasized how important focus control is in soft tissue and skin infections complicated with abscesses. It should be noted that empiric piperacillin/tazobactam monotherapy has been associated with an increased mortality in patient with bacteremia caused by *E. lenta* [16]. Therefore, piperacillin/tazobactam monotherapy should be avoided as empiric treatment in infections caused by both anaerobic microorganisms.

In addition to a correct antibiotic coverage guided by antimicrobial susceptibility, control of the focus by drainage of associated collections is essential in most of these infections [17]. The goal of source control is to eliminate the source of infection, control ongoing contamination, and restore premorbid anatomy and function.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest

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