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Scientific letter

Bacteremia due to *Desulfovibrio porci*. Novel anaerobic pathogen associated with infectious enterocolitis

Bacteriemia por Desulfovibrio porci. *Nuevo patógeno anaerobio asociado a enterocolitis infecciosa*

Microorganisms of the genus *Desulfovibrio* are strictly anaerobic, curved and spiral-shaped gram-negative bacilli, with the capacity to reduce sulfur compounds to hydrogen sulfide. They are present in the environment and are part of the flora of the human gastrointestinal tract.[1] Rarely, cases of primary bacteraemia (intestinal translocation) and intra-abdominal infections have been described, usually due to *Desulfovibrio desulfuricans (D. desulfuricans)* and *Desulfovibrio fairfieldensis*.[2] They are very slow-growing microorganisms in anaerobiosis and have intrinsic resistance to beta-lactam antimicrobials, including piperacilin/tazobactam.[3] *Desulfovibrio porci (D. porci)* is a new species recently described (https://lpsn.dsmz.de/species/desulfovibrio-porci) in the gastrointestinal tract of pigs,[4] with no reported cases of infections in humans.

We present two cases of bacteraemia due to *D. porci* in patients with enterocolitis and sepsis.

Case 1. 65-year-old woman with a liver transplant treated with mycophenolate and tacrolimus, who came to Accident and Emergency in August 2023 after 2–3 days of diarrhoea without blood or mucus, and vomiting, with a history of liver transplant in December 2014. Examination revealed that she had no fever, but she was dehydrated and had tachypnoea. Her abdomen was soft and depressible, but at the same time she had crampy abdominal pain and oligoanuria, with no evidence of peritonism, metabolic acidosis, or acute kidney injury. On CT there were clear signs of enterocolitis. Fluid therapy and antibiotic therapy with piperacilin/tazobactam were started in Accident and Emergency. The patient developed septic shock of abdominal origin, being admitted to the ICU with a poor response, with multiple organ failure, leading to her death less than 24 h after her admission to hospital. Her condition was diagnosed as septic shock due to infectious enterocolitis.

Case 2. A 64-year-old man, with no relevant history, who attended Accident and Emergency in January 2022, due to abdominal pain and diarrhoea without blood or mucus of five days' duration, an episode of self-limited rectal bleeding, and acute kidney injury. He did not report fever. On examination, diffuse abdominal pain was observed, without active bleeding on rectal examination, dehydration, tachypnoea, distended abdomen without peritonism, anuria and metabolic acidosis. On CT there were clear signs of enterocolitis. The patient developed septic shock of abdominal origin. He responded poorly to treatment with piperacilin/tazobactam, with refractory hypotension, anuria, haemodynamic and cardiac failure, and died in less than 24 h, despite antibiotic treatment under emergency observation, due to refractory septic shock and enterocolitis.

In both cases, in blood cultures obtained in Accident and Emergency (BACTEC FX system, Becton–Dickinson, two sets consisting of an aerobic bottle, BACTEC Plus Aerobic/F and one anaerobic, BACTEC Lytic/10 Anaerobic/F) growth was detected in an anaerobic flask on day 5 of incubation, with curved gram-negative bacilli being observed in the Gram stain, with little affinity for the counterstain. They were subcultured on Columbia blood agar and chocolate agar plates (ThermoFisher) at 35 °C in an aerobic atmosphere with 5% of CO_2 and Schaedler plates (ThermoFisher) at 35° in an anaerobic atmosphere (GENbox anaer, bioMérieux), with growth on the anaerobiosis plates of very small punctate, transparent, smooth-edged, non-haemolytic colonies after 72 h of incubation. Identification with mass spectrometry (MALDI-TOF, Bruker) did not provide any results.

In both cases, a microbiological study of the faeces was performed, in one bacteriological culture and in the other molecular detection by multiplex PCR (QIAstat-Dx Gastrointestinal Panel 2, Qiagen), with no pathogens detected.

For taxonomic assignment, the strain was sent to the Laboratorio de Referencia e Investigación en Taxonomía [Reference and Research Laboratory in Taxonomy] Centro Nacional de Microbiología [National Microbiology Centre], Instituto de Salud Carlos III [Carlos III Health Institute]. The identification of the strains was carried out by analysis of the *16S rRNA* gene sequence using E781 and U1115 as primers and the Applied Biosystems 3730xl DNA Analyzer. For strain CNM20220034 (case 1), a 1304 bp sequence was obtained which, when compared to the sequences of the type strains deposited in the GeneBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), had an percent identity of 99.2% with the type strain of *D. porci* (PG-178-WT-4). For strain CNM20231004 (case 2), a 1409 bp sequence was obtained with a percent identity of 99.2% (Fig. 1). The alignment of the sequences of CNM20220034, CNM20231004 and the type strain of *D. porci* using Clustal W (DNAStar Lasergene 15), shows a percent identity of 100% between the two strains.

The antibiogram was performed using the epsilometric method (E-Test, bioMérieux) on *Brucella* agar with H & K only in the first case, detecting resistance to penicillin (MIC > 32 mg/l), amoxicillin/clavulanic acid (MIC > 256 mg/l), piperacilin/tazobactam (MIC > 256 mg/l) and sensitivity to imipenem (MIC = 1 mg/l), clindamycin (MIC = 0.5 mg/l) and metronidazole (MIC ≤ 0.016 mg/l). The CLSI criteria (M100, Ed. 33) were followed for the clinical interpretation of MIC.

The two cases were patients with severe enterocolitis who were admitted to hospital, developing septic shock and dying within less than 24 h. Antimicrobial treatment with piperacilin/tazobactam, very common in intra-abdominal infections, would not be suitable for infections due to *Desulfovibrio* spp., which is intrinsically resistant to it.

For the diagnosis of infections caused by *Desulfovibirio* spp., it is important to remember the need to extract blood culture bottles in anaerobiosis, in the case of enterocolitis, incubate them for

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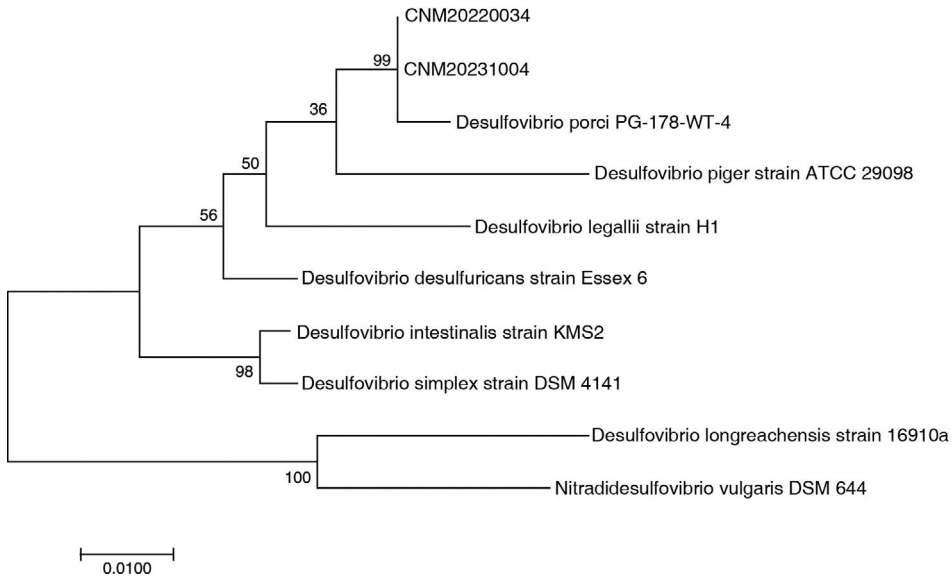

Fig. 1. Phylogenetic tree made with the 16S rRNA sequences (1245 bp) of the strains CNM20220034 (case 1) and CNM20231004 (case 2) and with respect to the type strains of the different related species of the genus *Desulfovibrio* (MEGA7, method Maximum Likelihood based on the Tamura-Nei model, 1000 replicates, branch length calculated based on the number of substitutions per position). The numbers on the branches indicate the frequency of grouping of the taxa.

at least five days, perform Gram staining on all blood culture bottles with growth detection, leave the agar plates in incubation for 72 h in anaerobic atmosphere after having observed the microorganisms in the staining, and perform the taxonomic assignment using molecular targets in the microorganisms not identified by usual methods (for example, biochemical tests, mass spectrometry). Added to that, commercial multiplex PCR techniques would not identify these microorganisms either because they do not have them included in their targets.

D. porci is an anaerobic microorganism, which is very slow and difficult to detect in blood cultures, which could be involved in severe cases of infectious enterocolitis with sepsis. The clinical implication of this microorganism could be underestimated if blood cultures are not processed correctly. This is because it is slow to grow in liquid and solid media, difficult to visualise in Gram stain and difficult to identify, as it is not included in the databases of the commercial systems, requiring the application of molecular targets with taxonomic value such as the *16S rRNA gene*.

In general, bacteraemia due to microorganisms of the genus *Desulfovibrio* is caused by the species *D. desulfuricans* and *D. fairfieldensis*, due to bacterial translocation from the gastrointestinal tract in patients with colon disease, with a good outcome in most cases.[5]

In our cases, *D. porci* bacteraemia was associated with enterocolitis (without being able to determine whether the *D. porci* infection was the cause of the enterocolitis, or the bacteraemia was a consequence of the intestinal disease), severe sepsis and the death of the two patients due to septic shock.

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