Diagnosis and treatment challenges in a rare *Clostridium* infection: A case report

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Abstract. The *Clostridium* genus includes >180 species of Gram-positive, anaerobic, sporulating bacteria. Under certain conditions, these can cause a wide range of invasive infections in humans. *Clostridium paraputrificum* occurs in the commensal intestinal flora and related bacteremia typically occurs secondary to an injury to the intestinal mucosa and in the presence of predisposing conditions, such as gastrointestinal disorders, malignancies, diabetes, HIV infection or neutropenia. The current study presents the case of a 70-year-old male patient, a rural resident living in poverty, with a history of alchohol consumption and cardiovascular pathology. Several initial and subsequent diagnoses were ruled out by successive investigations (e.g., stroke, meningitis, localized tetanus). Blood cultures were eventually found positive for *Clostridium paraputrificum* and the patient developed septic shock despite treatment with metronidazole and penicillin G. Once switched to carbapenem, the patient progressed favorably, suggesting that carbapenem could work as a first-line antibiotic treatment for *Clostridium paraputrificum* infections.

Introduction

The genus *Clostridium* is comprised of Gram-positive, anaerobic, spore-forming bacteria. It is one of the largest groups of bacteria, with >180 species capable of causing bacteremia and a variety of invasive infections in humans (1). The most common species of this genus is *Clostridium perfringens* (42%), followed by *C. septicum* (14%), *C. ramosum* (9%), *C. clostridioforme* (6%) and *C. difficile* (5%). By contrast, *C. paraputrificum* has been identified in only 1% of cases, which may explain why its clinical significance has not been fully elucidated, yet (2).

C. paraputrificum is a participant in the generally harmless commensal intestinal flora. As with other such species, bacteremia can be triggered and cause injury to the intestinal mucosa, facilitated by underlying diabetes, malignancies, HIV infection, alcohol consumption, gastrointestinal disorders or neutropenia (3,4). The most frequently reported conditions with *C. paraputrificum* as an etiological agent are sepsis, liver abscess, septic arthritis, osteomyelitis, aspiration pneumonia, acute necrotizing enterocolitis and colonic necrosis (1,5,6).

Clostridia appear as gram-positive, typically large, straight or curved rods, with slightly rounded ends, measuring ~2-6 μm in length and 0.5-1 μm in diameter. Spores are oval and subterminal or terminal. Pleomorphism forms are also common: Most species of *Clostridium* are motile with peritrichous flagella (7). A particular strain, M-21, has the capacity to produce gas by using chitin and glucose (8). In the present case, Gram-positive anaerobic rods with spores were observed on the Gram-stained blood cultures, and these were eventually identified as *C. paraputrificum*.

The genome of *C. paraputrificum* consists of a single circular chromosome. Certain strains may carry plasmids, which are extrachromosomal DNA that can confer additional traits, such as antibiotic resistance. 16S rRNA gene sequencing is used for identifying *C. paraputrificum* (7).

The current study reported the case of a 70-year-old patient with sepsis followed by septic shock caused by *C. paraputrificum*, whose aggravating condition was likely facilitated by a social background of poverty and alcohol consumption rather than by pre-existing conditions previously associated with this rare bacterial infection.

Materials and methods

Identification of bacteria. To test for suspected bacterial infections, blood culture vials were incubated in a BD BACTEC FX Blood Culture system (BD Biosciences) for seven days according to hospital and laboratory protocols. After 48 h of incubation, one of the two blood cultures showed the growth of bacteria. The identification of the bacteria was achieved with matrix-assisted laser desorption ionisation

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(MALDI)-time-of-flight technology (MALDI Biotyper; Bruker Daltonics), which is used to sequence proteins, map biomolecules in tissues, identify microorganisms and to analyze several thousand biochemical assays in a day (9). Gram staining was also performed, as well as various cultures from urine and bed sores secretions to screen for multi-drug resistant bacteria. The test was performed according to standard procedures.

Toxins A and B for *Clostridioides difficile* were investigated by means of immunochromatographic assay using a rapid test kit supplied by DDDiagnostic. The stool sample was processed to obtain a suspension (a small amount from the stool sample was mixed with the buffer solution). This was then exposed to specific antibodies for *Clostridioides difficile*'s A and B toxins for 10-20 min, after which the results were displayed on the test kit. A control line appeared, confirming that the test was performed correctly, and the presence of toxins would have been indicated by additional lines.

Antibiogram methodology. For the antibiogram, the method of microdilutions in broth (Micronaut system) on Bruker plates was used. The specificity of this method was 99% according to the laboratory staff and other published research (10). Genetic assays could not be performed, as our laboratory is not equipped with the necessary kits.

Case report

A 70-year-old male patient was first brought to the emergency room of 'Dr. N. Oblu' Emergency Neurology Hospital (Iași, Romania) in February 2024. The patient's symptoms had started ~3 days prior and on admission, the patient's general condition was deteriorating, with apparent confusion and inability to maintain orthostatism. The patient was an impoverished rural resident with a history of cardiovascular disease (heart failure) and chronic alcohol consumption.

On admission, the patient was presenting with signs suggestive of an ischemic stroke, yet the brain CT scan showed no evidence of stroke or recent hemorrhage (data not shown). Instead, the chest CT revealed areas of alveolar condensation affecting the medium lobe of the right lung almost entirely (aspect suggestive of lobar pneumonia), as well as in posterior, basal, bilateral areas, including left pleurisy of ~10 mm and pulmonary scleroemphysema (data not shown). These results, together with the neurological examination, excluded a neurological emergency, and other possible causes were also excluded based on further specialized investigations (cardiology, gastroenterology, pneumology, nephrology). It was not possible to include CT images, as CT scanning is a service outsourced to another hospital, and the results were received as already interpreted.

Paraclinical tests indicated leukocytosis [leukocytes, 33,020/mm^3 (normal range, 6,000-13,500/mm^3)] with neutrophilia [neutrophils, 92.6% (normal range, 30-75%)], C-reactive protein, 317 mg/l (normal range, <0.5 mg/l), coagulation disorders and hepatocytolysis [alanine aminotransferase, 351 U/l (normal range, 5-40 U/l); and aspartate aminotransferase, 143 U/l (normal range, 5-37 U/l)], as well as evidence of nitrogen retention expressed as high serum urea [158 mg/dl (normal range, 15-50 mg/dl)] and elevated creatinine [4 mg/dl (normal range, 0.6-1.1 mg/dl)] (Table I). On clinical examination, the patient presented muscle hypertonia and a stiff neck. The suspicion of meningitis was thus raised and the patient was subsequently transferred to the 'Sf. Parascheva' Clinical Hospital of Infectious Diseases (Iași, Romania).

On arrival, the patient was afebrile, hemodynamically stable, with slightly elevated blood pressure [123/86 mmHg (normal range, <120/80 mmHg)] and heart rate [100 bpm (normal range, 60-90 bpm)], and with low oxygen saturation [90% (normal range, 95-100%)], and this was addressed with oxygen therapy (4 l/min). The cardiopulmonary auscultation revealed crackling rales on the right hemithorax and diminished vesicular murmur on the left. The patient's abdomen was depressed, painless on deep palpation and diuresis was present. In addition, grade I calcaneal and grade I-II sacral eschars were noted, while mucous membranes were normally colored. The patient was conscious, able to open his eyes and perform eye tracking, and able to use his limbs spontaneously. However, he became uncooperative and his condition appeared to be deteriorating ~6 h after admission.

A lumbar puncture was performed and it excluded an inflammatory reaction. Considering the polymorphic symptomatology and the general condition of the patient, the initial therapeutic intention was to address a wide spectrum of bacteria. Thus, treatment was initiated with ciprofloxacin 200 mg and imipenem 500 mg (taking into account creatinine levels when deciding on these dosages), analgesic and antipyretic medication, a gastric protector and hydro-electrolytic rebalancing infusions. Furthermore, the patient's cultures for multi-drug resistant bacteria were negative.

The patient was monitored and his general condition continued to aggravate over the next 24 h. The patient's oxygen needs increased to 10 l/min and creatinine levels rose to 6 mg/dl, a sign of nitrogen retention suggestive of deteriorating kidney function. In addition, the clinical observation of a rigid (wooden) abdomen was made. A contrast CT of the abdomen and pelvis was performed at the 'Sf. Spiridon' Emergency Clinical County Hospital (Iasi, Romania). A surgical consultation was also provided, yet neither revealed any evidence that would justify the need for emergency surgery (e.g., no perforated gastric ulcer or mesenteric ischemia). Considering also the patient's accelerating intestinal transit, he was tested for toxins A and B of *Clostridioides difficile,* with negative results. The test was performed using immunochromatographic assay.

Taking into account the clinical sign of 'wooden abdomen', in the absence of changes detectable by CT imaging, the suspicion of localized tetanus was raised. By this time, three days after admission, excoriations with grating lesions, bed sores at the lumbar-sacral level and 'diaper' dermatitis had been noted by visual examination, and these were considered possible gateways for such an infection.

At four days after admission, due to creatinine increasing further to 6.9 mg/dl under the initially established therapy, the patient was transferred to the Nephrology Department of 'Dr C. I. Parhon' Clinical Hospital (Iasi, Romania) where a dialysis session was initiated. Serological analysis for *Clostridium tetani* was performed and the result was negative. After localized tetanus was ruled out, the patient's earlier blood samples (collected prior to the initiation of antibiotic therapy)

Table I. Evolution of biological parameters.

Item (normal ranges)	1st day	2nd day	3rd day	4th day	5th day
Blood count					
WBC, mm^3 (4,000-10,000)	18,140	-	-	-	11,530
NEUT, % (45-80)	94.2	-	-	-	85.7
LYMPH, % (20-45)	2.4	-	-	-	4.4
HB, g/dl (13.2-17.3)	12.7	-	-	-	10.7
HCT, % (39-51)	37.5	-	-	-	321
PLT, mm^3 (150,000-380,000)	340,000	-	-	-	197,000
Inflammatory markers					
CRP, mg/l (0-5)	375.68	195.62	-	-	-
Procalcitonin, ng/ml (<0.5)	>10	-	-	-	-
Fibrinogen, g/l (2-4)	4.66	-	-	-	-
Biochemistry					
Urea, mg/dl (15-50)	170	235	277	293	227
Creatinine, mg/dl (0.6-1.1)	3.95	5.18	5.99	7.25	4.84
Total protein, g/l (67-87)	56.64	-	-	-	50.98
Liver markers					
ALT, U/l (5-40)	150	-	-	111	65
AST, U/l (5-37)	348	-	-	152	111
Total bilirubin, mg/dl (0.2-1)	0.65	-	-	-	0.40

WBC, white blood cells; NEUT, neutrophils; LYMPH, lymphocytes; HB, hemoglobin; HCT, hemaotocrit; PLT, platelets; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

were further processed and *Clostridium paraputrificum* was identified as gram-positive rods with spores on Gram-stained cultures, as illustrated in Figs. 1 and 2.

After establishing the etiology from the blood cultures, the resumption of diuresis and the decrease in creatinine to 4.25 mg/dl, the patient was transferred to an intensive care unit for patients with infectious diseases at the 'Sf. Parascheva' Clinical Hospital for Infectious Diseases (Iasi, Romania) five days after admission. Antibiotic sensitivity tests for anaerobic organisms showed that the *C. paraputrificum* strain was sensitive to meropenem, metronidazole, penicillin G, piperacillin/tazobactam, vancomycin, ampicillin, amoxicillin/clavulanic acid and imipenem (Table II). The treatment was changed to penicillin G and metronidazole.

At this point, ~6 h into the new antibiotic treatment, the patient's general condition continued to deteriorate both neurologically and hemodynamically (hypotension). Septic shock was established, vasopressor support with noradrenaline was initiated and it was deemed necessary to change the antibiotic therapy to carbapenem (imipenem) and fluoroquinolone, in addition to intravenous hydro-electrolytic rebalancing. After three days of antibiotherapy with fluoroquinolone and carbapenem, the patient's general condition improved and he was moved from intensive care to a regular infectious diseases ward, with the indication for further treatment and continuous monitoring. In total, the patient was under this treatment for 10 days and standard therapy was also provided according to the symptoms during this time. Eventually, the patient had a favorable outcome.

Initially, despite the fact that the patient was under appropriate antibiotics therapy started based on the antibiogram results, his evolution was not favourable. For this reason, it was assumed that the patient may be superinfected with a multi-drug resistant nosocomial bacterium using his bed sores as a gateway. After changing the antibiotherapy to carbapenemes and floroquinolones, the patient's condition finally began to improve. Of note, the patient's cultures for multi-drug resistant bacteria were negative, but this does occur and such results do not invalidate the existence of these bacteria. Clinicians are trained to assess the patient's signs, symptoms and laboratory results globally, which in this case proved life-saving. The patient was clinically reassessed seven days after discharge, when the results were satisfactory, and the liver and renal functions were back to normal. This was the patient's final examination in our hospital, after which further monitoring and care were provided by the patient's general practitioner, as needed.

Discussion

The gastrointestinal tract hosts a multitude of bacteria that form the intestinal microbiota. *Clostridium* species dominate this commensal, normally innocuous microflora, ensuring intestinal homeostasis, yet they also have the potential to cause serious harm. Case reports discussing infection with *C. paraputrificum* specifically are scarce in the literature. Only 14 such cases were described between 1961 and 2023, one implication being that related mortality cannot be accurately established (2,4).

Table II Antimicrobial susceptibility test for *Clostridium paraputrificum*.

Antibiotic	Minimum inhibitory concentration, mg/l	Sensitivity
Clindamycin	0.5	Resistant
Meropenem (carbapenem)	≤0.5	Sensitive
Metronidazole	1	Sensitive
Penicillin G	0.5	Sensitive
Piperacillin/Tazobactam	≤1/4	Sensitive
Vancomycin	≤2	Sensitive
Ampicillin	0.5	Sensitive
Amoxicillin/clavulanic acid	<0.5/0.25	Sensitive
Imipenem (carbapenem)	<0.5	Sensitive

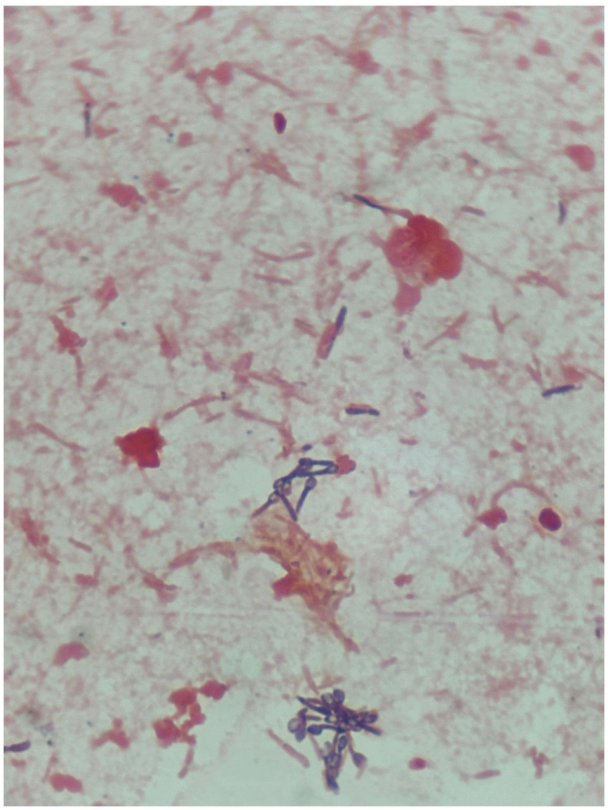

Figure 1. Microscopic identification of *Clostridium paraputrificum* (magnification, x1,000).

Figure 2. Plate with the culture of *Clostridium paraputrificum* (without stain).

C. paraputrificum has been identified as a source of infection in patients with AIDS (1), colon necrosis (1), intestinal neoplasm (2,3), appendicitis (4), liver abscess (5), and septic arthritis (6). One peculiarity of the present case was that the abdominopelvic CT with contrast excluded gastrointestinal causes, unlike most other cases published so far (1-5). Usually, risk factors for this particular infection are malignancy, HIV infection, diabetes, alcohol consumption and gastrointestinal pathology (1). Poor living conditions were the more likely contributors to the patient's bacterial infection in the present case, possibly enhanced by alcohol consumption. Regarding the patient's history of heart failure, it was not considered a risk factor for this particular infection, although future case reports may provide evidence to suggest otherwise.

With regard to treatment options, most studies describe the resistance of *C. paraputrificum* to clindamycin (1,2,4). Instead, previous cases reported by others showed 99% of the isolates were sensitive to metronidazole and 90% sensitive to penicillin, which suggests that the initiation of empirical treatment should include metronidazole to reduce the risk of therapeutic failure.

In the case presented by Intra *et al* (2), the patient had a substantial gastrointestinal neoplasm (the pathology most related to *C. paraputrificum* infection) and the isolated strain was resistant to penicillin G. In a case reported by Mostel *et al* (4), the patient was treated with ampicillin-sulbactam and the evolution was good. Related to our experience, this suggests that the sensitivity of *C. paraputrificum* may differ among regions and may also depend on the patient's comorbidities.

Another interesting aspect is that, usually, such bacteria are not isolated in the hemoculture, and even when clostridial infections are specifically considered, *Clostridioides difficile* is the most common culprit and just 1% of cases are due to *Clostridium paraputrificum* (2). Establishing a definitive diagnosis proved a formidable challenge for us, as no such case had been previously encountered at our department. The patient's state aggravated despite receiving antibiotic treatment that should have been effective according to the antibiogram, so a nosocomial infection caused by multi-drug-resistant skin bacteria would have appeared more likely,

considering the patient's bedsores and the hospitalization period.

It is noteworthy that the patient of the present study was economically impoverished and living in precarious conditions, while also being a long-term alcohol user. A social background of poverty and hardship can have substantial implications on health, including a compromised immune system facilitating infection with bacteria that only rarely affect patients with certain predisposing pathologies. Our sharing of this case with the scientific community is, in a sense, an open invitation to assess the role of socio-economic factors in rare bacterial infections.

In conclusion, *C. paraputrificum* is an uncommon species of *Clostridium* and it is rarely evidenced in patients' cultured samples. The present study reported an unusual case of *C. paraputrificum* infection in a patient who developed septic shock while under therapy with penicillin G and metronidazole. To the best of our knowledge, this is the first reported case without any associated gastrointestinal pathology, and where a social background of poverty may have been a risk factor instead.

Prior reviews and studies recommend metronidazole as empirical treatment (1-6), yet the patient of the present study did not show any improvement while on it, so metronidazole may not be effective in every case. Switching to carbapenem produced good outcomes, including recovery from shock and normalization of renal function, mental status and mobility, suggesting that carbapenem could be considered as the first line of treatment in similar cases. Further research is needed to establish the impact of bacteremia with *C. paraputrificum*, as well as the definition of the pathogenesis and the investigation of risk factors.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization: CM, CEF and BBM; methodology: AR and CEF; software: AR; validation: CEF, BBM, ERIB and CM; investigation: CEF, EM, BBM and LM; resources: LM; writing-original draft preparation: CEF and BBM; writing-review and editing: CM, MO and CEF; visualization: MO and CM; supervision: MO and CM. BBM and CEF confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The patient provided written informed consent to participate in this study.

Patient consent for publication

The patient provided written informed consent for the publication of this case report and all accompanying images.

Competing interests

The authors declare that they have no competing interests.

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