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CLINICAL CASE

Septicemia due to *Bacillus clausii* after the use of probiotics. A complication to keep in mind

Septicemia por *Bacillus clausii* posterior al uso de probióticos. Una complicación para tener presented

Michael Muñoz[a], Elizabeth Castaño G.[b], Raúl Esquivel Suman[b], Manuel Alvarado[c]

[a]Residente de Pediatría. Universidad del Sinú. Montería, Colombia.
[b]Servicio de Infectología, Hospital del Niño Dr. José Renán Esquivel. Panamá, República de Panamá.
[c]Sala de Cuidados Intensivos Pediátricos. Hospital del Niño Dr. José Renán Esquivel. Panamá, República de Panamá.

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What do we know about the subject matter of this study?

Probiotics are live microorganisms that benefit the host in different clinical situations. Bacillus clausii is frequently used, but potential complications secondary to this agent in pediatric patients are little known.

What does this study contribute to what is already known?

This case report discusses the risk factors for bacteremia and/or sepsis due to the use of the probiotic Bacillus clausii in a pediatric patient, highlighting malnutrition, immunosuppression, and intestinal epithelial damage due to severe diarrhea.

Abstract

Probiotics are live microorganisms that benefit the host in different clinical situations. *Bacillus clausii* is one of the most frequently used, but it is not without risk. To date, there are few reports of complications secondary to this agent in pediatric patients. **Objective**: To describe the case of an infant who developed after treatment sepsis due to *Bacillus clausii.* **Clinical Case**: A 4-month-old female infant of indigenous ethnicity, from a rural area in the interior of Panama, 3 hours away from the nearest health sub-center by canoe, and with protein-calorie malnutrition, presented with acute diarrhea and moderate-severe dehydration, receiving Enterogermina as part of the initial treatment. She was transferred to a tertiary hospital, where she arrived with impaired consciousness, respiratory distress, and signs of shock. The initial blood culture reported growth of methicillin-resistant *Staphylococcus aureus* (MRSA), the gastrointestinal panel was positive for *Clostridiodes difficile*, and later serial blood cultures of peripheral blood and central venous catheter confirmed growth of *Bacillus clausii.* With a torpid evolution and resistance to multiple antibiotic schemes, she died due to multisystem organ failure twelve days after admission. **Conclusions:** The use of probiotics as concomitant treatment in patients with some degree of immunosuppression should be administered with caution, considering the presence of risk criteria for complications such as malnutrition or intestinal epithelial damage due to severe diarrhea since they predispose to the development of bacteremia and/or sepsis.

Keywords:
Bacillus clausii;
Protein-Calorie Malnutrition;
Sepsis;
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Correspondence:
Michael Muñoz
md.maicol@gmail.com

Elizabeth Castaño G.
chabecast@yahoo.es

Edited by:
Luisa Schonhaut Berman

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Introduction

According to the World Health Organization (WHO), probiotics are defined as "live microorganisms which, when properly administered, have a positive impact on the health of the individual"[1]. They must meet certain characteristics, such as absence or very low virulence, and be classified as "generally safe" by the U.S. Food and Drug Administration (FDA) and as "qualified presumption of safety" by the European Food Safety Authority (EFSA), as well as being capable of passing through the digestive tract and having studies and/or clinical trials that certify their use and safety[1]. The EFSA presumes that *Bacillus clausii* (*B. clausii*) O/C (CNCM I-276), N/R (CNCM I-274), SIN (CNCM I-275), and T (CNCM I-273) contained in Enterogermina® is safe and has been added to the Qualified Presumption of Safety (QPS) list[2].

Bacillus clausii is a Gram-positive, motile, spore-forming bacillus bacterium that is currently one of the microorganisms used as a probiotic in the context of infectious diarrhea or secondary to antibiotic use in many countries worldwide[3]. Enterogermina® (Sanofi-Aventis S.p.A.), a spore-based compound, is a probiotic registered as a pharmaceutical preparation in 1958[4] and with OTC status since 1999[2]. It contains spores of four antibiotic-resistant *Bacillus clausii* strains, O/C (CNCM I-276), N/R (CNCM I-274), SIN (CNCM I-275), and T (CNCM I-273) and is recommended to restore intestinal microbial balance, particularly during antibiotic treatment[4]. Currently, there are two different formulations, freeze-dried capsules, and liquid vials, and are marketed in 55 countries around the world for the treatment of gut dysbiosis and the prevention of gastrointestinal infectious diseases[3].

Abbrescia et al.[5] demonstrated that *B. clausii* has intrinsic resistance genes to different classes of antibiotics, such as cephalosporins, macrolides, and aminoglycosides, which could pose a problem in the future since it has been demonstrated that bacteria can share resistance genes through plasmids, transposons, and integrons. Since probiotics are widely used, it should be considered that they can lead to significant infection in patient subgroups such as those immunosuppressed[6]. However, there are few described cases of complications secondary to this agent in pediatric patients.

The objective of this work is to describe the case of an infant who developed *Bacillus clausii* sepsis after probiotic treatment, in order to alert about a potential complication that is not well known in undernourished or immunosuppressed patients.

Clinical Case

A 4-month-old indigenous infant from Urracá, a rural area in the interior of Panama, 3 hours away from the nearest health sub-center by canoe. Her history highlights that she was the fourth child, born by vaginal delivery at home attended by a family member, without prenatal check-ups; weight, length, and Apgar score at birth are unknown. She was not breastfed and was fed with powdered starter milk formula with iron for children under 6 months, receiving 3 ounces every 4 hours.

The nuclear family consisted of 6 people (parents and 4 children) who lived in a two-room house with board walls and floor and a palm leaf roof. They had no electricity, having to resort to kerosene lamps for lighting, drew water from a well, disposed of their waste in the river, and burned garbage. Their income came from subsistence agriculture.

When she was 4 months, she had not received any health care and did not have the vaccines included in the national expanded program of immunizations. According to her parents, her neurodevelopment was normal until her hospitalization.

The child visited a healthcare center due to a 4-day history of diarrhea, without mucus or blood, associated with vomiting after being fed (the mother gave her tea because she could not tolerate milk), no fever, and without respiratory symptoms. Oral fluids and 4 doses of Enterogermina® (*B. clausii*: two billion spores/5 mL) were administered. Due to a lack of supplies (there were no catheters or intraosseous catheters for the administration of intravenous fluids), she was transferred to a second-level hospital in the capital of the province and then to our institution in Panama City with a diagnosis of acute gastroenteritis and severe dehydration.

She arrived at the emergency department with altered state of consciousness and dehydration characterized by crying without tears and dry oral mucosa. She presented with +++ edema in her hands, feet, abdomen, and face. She was afebrile with signs of shock, capillary refill > 2 seconds, cold extremities, thready pulse, and cutis marmorata, heart rate 170 bpm, respiratory rate 55 bpm, blood pressure 91/37 mmHg, and oxygen saturation 99%. At admission, weight was 4.7 kg, length 56 cm, and Z-score length/age -2.52; it was not possible to quantify the Z-score of weight/length and weight/age due to severe dehydration. On segmental examination, there were fine rales in both lung basal lobes, erythemato-squamous lesions with desquamation, and others with hypopigmentation on the trunk and upper limbs (interpreted as pellagra).

A Ringer's lactate bolus at 10 ml/kg dose was indicated in the emergency department, and she continued

with 5% dextrose in 0.33% saline solution 500 ml infusion at 29 ml/hr in 6 hours without KCL until diuresis was obtained. Due to suspicion of sepsis, she received Ceftriaxone at 50mg/kg/day, then was stabilized and sent to the ward where she continued with fluid management receiving 500 ml of 5% Dextrose in 0.9% saline at 20 ml/hr.

Among the tests, a complete blood count revealed leukocytosis 39.0x103/uL, severe anemia 5.6 g/dL, and thrombocytosis 502 x 103/uL. Table 1 shows the rest of the results. She received a transfusion with 50 ml of filtered and leukoreduced red blood cells and 40 cc of fresh frozen plasma due to altered clotting times (table 1). The enteral feed was started by nasogastric tube and the infusion of Dextrose 5% in Saline 0.9% 500 cc was decreased to 15 ml/h, but she continued with negative water balance.

On the 2nd day, the initial peripheral blood culture reported positive with Gram-positive cocci in clusters, therefore, Oxacillin was added at 200 mg/kg/day, and Ceftriaxone dose was increased from 75 to 100 mg/kg/day; total fluids were increased to 120 cc/kg/day and calcium was corrected (received value 6.38 mg/dL).

On the 3rd day, she lost venous access, so a cen-

Table 1. Laboratory tests results during patient follow up

Tests	Admission	3rd day after admission	6th day after admission	12th day after admission	Reference values
Hemoglobin	5.6	10.4	9.1	6.5	10.5 - 14.0 g/dL
Hematocrit	17	34.2	29.5	21.1	32 - 42%
Platelets	502	154	54	79	150 - 450 x10^3/ uL
Leucocytes	37.4	22.6	39.0	35.0	6 -14x 10^3/ uL
Neutrophils	79	91	76	82.2	20% - 40%
Lymphocytes	15	5	16	13.5	50% - 60%
Coagulation times					
Partial Thromboplastin time	> 60	38.3	37.9	> 120	21.9 - 40.8 seg
Prothrombin time	32	27.1	21.9	34.4	12.1 - 17.7 seg
Fibrinogen	86	182.6	181	72	275 - 395 mg/dl
INR	2.55	2.08	1.60	2.94	0.83 - 1.12
Chemistry					
Alkaline Phosphatase	232	208	172	131	80 - 345 U/L
Aspartate amine transferase	64	243	591	227	20 - 63 U/L
Alanine amine transferase	16	129	185	200	12 - 37 U/L
Total bilirubin	0.92		1.63	3.02	0.0 - 1.0 mg/dL
C reactive protein	5.49	4.32	2.97		0.05 - 0.79 mg/dL
Sodium	133	137	147	132	134 - 142 mEq/L
Calcium	7.2	6.52	4.86	8.67	7.9 - 10.7 mg/dL
Creatinine	0.44	0.39	0.79	1.31	0.20 - 0.40 mg/dL
Blood urea nitrogen	2.1	2.4	7.6	12.2	4.0 - 14.0 mg/dL
Total protein	4.8	2.91	3.42		3.9 - 7.6 g/dL
Albumin		1.3	1.3		2.2 - 4.4 g/dL
Cardiac Panel					
MB Creatin kinase			400		0-16 ng/mL
Troponin I			2.66		0.120 ng/mL
N-terminal brain natriuretic peptide			> 30.000		27 - 265 pg/mL
Myoglobin			> 2.000		61.5 ng/mL in females

Source: Hospital del Niño Dr. José Renán Esquivel. Clinical Laboratories.

Table 2. Results of blood cultures during hospitalization and isolates

Date of Sample	Blood Cultures	Isolates
(Day 1)	Peripheral Blood	Methicillin Resistant *Staphylococcus aureus*
(Day 2)	Peripheral Blood	*Bacillus clausii*
(Day 4)	Central Venous Catheter Blood	*Bacillus clausii*
(Day 4)	Peripheral Blood	*Bacillus clausii*
(Day 8)	Central Venous Catheter Blood	*Bacillus clausii*

Source: Hospital del Niño Dr. José Renán Esquivel, Clinical Laboratories.

tral venous catheter (CVC) was placed. Since she appears hypoactive, with underhydrated oral mucosa, increased respiratory work, cold extremities, and capillary refill 3-4 seconds, Ringer's lactate dose was increased to 20 cc/kg in one hour; arterial blood gas showed uncompensated metabolic acidosis with pH 7.26, HCO_3 13mmol/L, PCO_2 28.4 mmHg, PO_2 39.2 mmHg, and lactate 2.8 mmol/L. The patient was intubated and transferred to the pediatric intensive care unit (PICU) where she was connected to mechanical ventilation.

Total fluids were administered at 100 cc/kg, adrenaline infusion, low-salt albumin, adding 10% calcium gluconate. Fentanyl was changed to remifentanil due to increased liver enzymes.

The blood culture on admission reported growth of methicillin-resistant *Staphylococcus aureus* (MRSA), Oxacillin was withdrawn, and Clindamycin was added at 40 mg/kg/day. Blood culture on the second day of admission to the PICU showed a positive smear for Gram variable bacillus, Ceftriaxone was changed to Ceftazidime at 150 mg/kg/day.

During the 1st day in the PICU, a significant increase in serum biomarkers of cardiac damage was observed (table 1), the echocardiogram showed mild mitral and tricuspid regurgitation, left ventricular dilatation, left ventricular ejection fraction (LVEF) 58%, with no evidence of thrombus, vegetations, or pericardial effusion, she was therefore diagnosed with acute myocarditis. Milrinone infusion at 0.4 mcg/kg/min, furosemide, and IV immunoglobulin 1 g/kg single dose were started.

In a blood culture performed on the second day, the germ was identified as *Bacillus clausii*, identified by the VYTEK® 2 system; the susceptibility profile was not performed because the equipment did not have cut-off points for this germ, thus antibiotic coverage was adjusted, considering it was not a contaminant, and Ceftazidime was changed to Ciprofloxacin at 30 mg/kg/day and Ceftaroline was added at 8 mg/kg every 8 hrs together with Clindamycin for MRSA. Subsequent 3 blood cultures at 48-hour intervals between each (table 2) were positive in both peripheral blood and CVC for isolation of *B. clausii* (figure 1).

On the 6th in the PICU, the results of the gastrointestinal panel (mariPOC® Gastro test) performed on the second day were received and detected *Clostridiodes difficile* toxin A/B; tests for *Campylobacter jejuni*, *Norovirus GI*, *Norovirus GII.4*, Adenovirus, and Rotavirus were negative. Given these findings, therapy was escalated to IV Vancomycin at 60 mg/kg/day dose, oral metronidazole was added, and ceftaroline, clindamycin, and ciprofloxacin were withdrawn, covering both *B. clausii*, *C. difficile*, and MRSA.

Tests for HIV, Chagas serological test, and SARS COV 2 antigen by immunofluorescence assay (IFA) were negative, and immunoglobulins were within normal range.

On the 7th day, she presented arterial hypertension, so spironolactone was added to the management.

On the 8th day, laboratory tests showed alterations in coagulation times and increase azotemia associated with 12 hours of anuria, however, due to the patient's condition, a peritoneal catheter was not placed, and vancomycin dose was adjusted and vitamin K was administered. The patient continued with anuria and anasarca and presented sustained hypotension, so noradrenaline was added but she evolved in a torpid manner, with multisystemic organ failure, and died after twelve days in the hospital. Necropsy was not performed because the mother refused authorization due to cultural reasons.

Discussion

The interaction between the intestinal microbiota and the host is the subject of current research. This can influence a large number of diseases and part of this research is focused on the development of creating modified bacteria/fungi with therapeutic use. These arrangements involve the probiotic stimulating or fa-

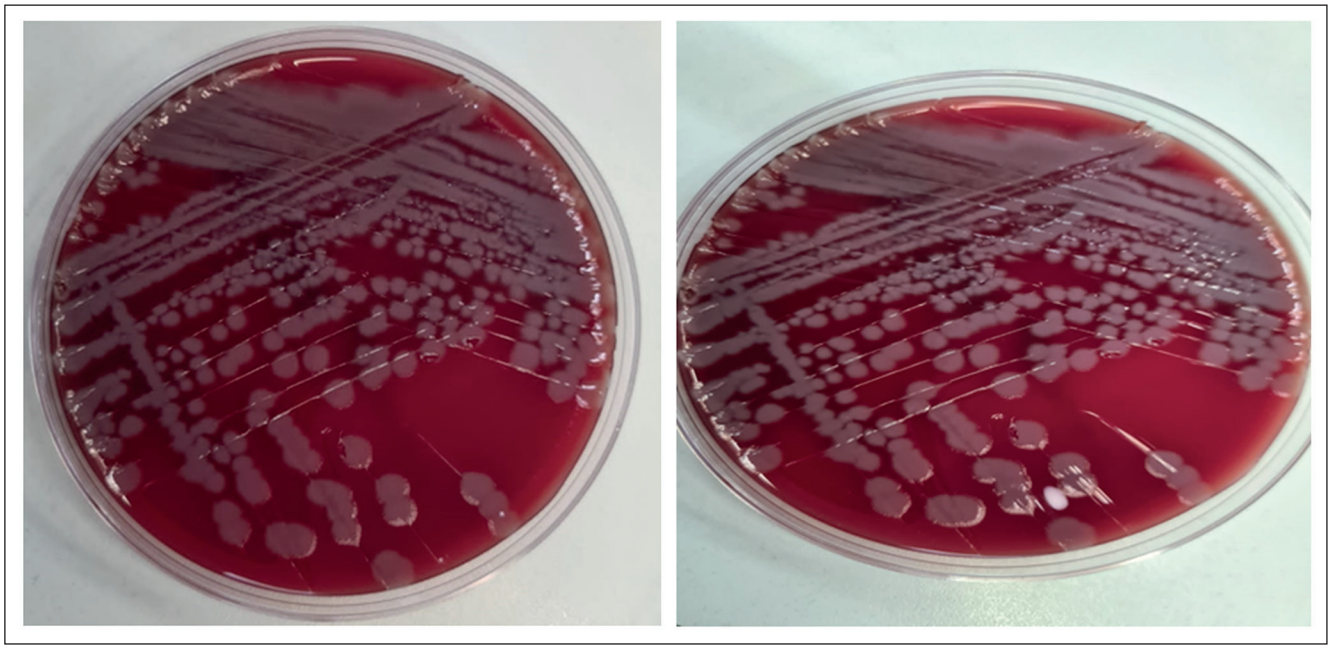

Figure. 1. Result of blood culture. Colonies of Gram-positive bacilli that grew in blood culture in solid medium "Agar with lamb's blood 5%". Microorganism identified: Bacillus clausii, using the VYTEK 2 system TM.

vorably modulating the host immune system, secreting antibiotic substances for virulent germs, or eliminating growth substrate for other pathogens[7].

The benefits of *B. clausii* for the intestinal microbiota have been reported, such as gene expression involved in the immune response, apoptosis, cell signaling, and modulation of cellular signaling[8]. This probiotic is indicated as a complementary treatment with oral rehydration solution for acute viral diarrhea. *B. clausii* can also be considered for the prevention of antibiotic-associated diarrhea, *Clostridiodes difficile*-induced diarrhea, and as an adjunct treatment for *Helicobacter pylori*, while in others, beneficial modulatory effects have been seen in allergic rhinitis and upper respiratory tract infections[2].

B. clausii survives transit in the intestine and maintains a considerable intestinal titer for up to 12 days after a single oral administration, which may have occurred in our patient causing the persistence of *B. clausii* bacteremia. *B. clausii* strains show different capacities to survive and persist, suggesting an adaptive strain to this environment, the spores are heat stable and can survive in environments with poor nutrition, dehydration, and low pH[9]. In healthy volunteers, *Bacillus clausii* was able to germinate, grow, and multiply in the gastrointestinal tract[2] which could explain the persistence of this bacterium in blood cultures.

Boyle et al.[10] proposed major and minor criteria to evaluate the risk of sepsis due to probiotics (table 3), in our case, the patient presented one major criterion (malnutrition), and three minor criteria, such as epithelial damage due to severe diarrhea, CVC, and coadministration of broad-spectrum antibiotics with resistance to probiotics, such as cephalosporins. The host with impaired immunity experiences difficulties in the suppression of exogenous bacteria, thus increasing the risk of sepsis using probiotics, such is the case of pediatric patients with acute malnutrition who, at the same time, have impaired intestinal epithelium by the same

Table 3. Clinical criteria to evaluate the risk of probiotic associated sepsis

Mayor Criteria
Immunodeficiency (includes malnutrition and cancer)
Premature newborn
Minor Criteria
Central Venous Catheter
Non competent intestinal epithelial barriers (severe diarrheic disease and intestinal inflammation)
Administration of probiotics through jejunostomy
Probiotic with high adhesion capacity to intestinal mucosae or with known pathogenicity.
Concomitant administration of broad-spectrum antibiotics to which probiotics are resistant
Valvular disease (only related ton *Lactobacillus*)

Source: Boyle et al.[10]

Table 4. Cases of *Bacillus clausii* bacteremia reported in medical literature.

Author	Year	Age	Patient profile	Treatment	Clinical evolution	Susceptibility
Gargar JD, Divinagracia RM[6]	2019	(3) Adults	Pneumonia-Stage IV Cancer Pneumonia- Stage IV cancer Ischemic colitis- Septic Shock	Not specified	Deceased Recovered Recovered	Not described
Joshi S et al.[12]	2019	5 months	Surgical Treated Heart Disease Malnutrition	Vancomycin	Deceased	Penicillin Vancomycin
Khatri AM et al.[14]	2021	17 months	Previously healthy	Ceftriaxone Ampicillin Gentamicina Levofloxacin Vancomycin Vancomycin VO Levofloxacin VO	Recovered	Ceftriaxone Levofloxacin Vancomycin
García JP et al.[15]	2021	87 years	COPD Hypertension	Vancomycin Cefepime Ampicillin Gentamycin	Recovered	Vancomycin, Ciprofloxacina
Princess I et al.[13]	2019	Medium age	Type 2 Diabetes Venous Brain Thrombosis	Teicoplanin	Recovered	Ciprofloxacina Vancomycin

mechanism, which greatly increases the occurrence of bacterial translocation into the bloodstream and the onset of sepsis.

Although the efficacy of probiotics has been proven in specific conditions, their use in children is massively extended due to their perception as innocuous products, however, recent evidence raises concerns about their safety in the pediatric population because serious opportunistic infections have been described after their use. D'Agostin et al.[11] conducted a review on invasive infections related to the use of probiotics in the pediatric population, finding a total of 49 cases reported from 1995 to June 2021, with the causative agents being *Lactobacillus spp*. (35%), *Saccharomyces spp*. (29%), *Bifidobacterium spp*. (31%), *Bacillus clausii* (4%), and *Escherichia coli* (2%), more than 80% were in children under 2 years of age, and 69.4% of the cases were associated with sepsis, as occurred in our case.

Undoubtedly, infections by *B. clausii* are exceptionally infrequent (table 4)[6,12-15] and are mainly observed in groups at higher risk that include patients with some type of immunosuppression, as occurs at the extremes of life, in neonates due to their immunological immaturity, and in the elderly due to their reduced immunity, in relation to certain diseases such as AIDS, neoplasms, or leukemia. In relation to primary or secondary malnutrition, the use of immunosuppressants in transplanted patients, with chronic diseases, including those requiring parenteral nutrition and with central intravenous catheters[4], Princess et al.[13] reported a case of bacteremia due to *B. clausii* sensitive to vancomycin and ciprofloxacin but resistant to penicillin (table 4), determined by E-test method based on Clinical and Laboratory Standard Institute (CLSI) standards[16]. However, this report mentions the difficulty of determining susceptibility to other antimicrobials due to the lack of cut-off points (table 3).

Conclusions

The administration of probiotics as concomitant treatment in patients with malnutrition or some degree of immunosuppression should be given with caution as part of the initial approach and should be considered if the patient presents criteria such as malnutrition or intestinal epithelial damage due to severe diarrhea, which predispose to the subsequent development of bacteremia or sepsis due to *Bacillus clausii*.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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