

A tale of caution: prolonged *Bacillus clausii* bacteraemia after probiotic use in an immunocompetent child

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Abstract

Introduction. *Bacillus clausii* as a probiotic supplement is increasingly used in both adult and paediatric patient populations. There is limited awareness about potential adverse effects.

Case presentation. We report a case of prolonged (111 days) *B. clausii* bacteraemia after brief probiotic use in a 17-month-old immunocompetent child, without a definite focus of infection and in the absence of predisposing risk factors or underlying co-morbidities. We identified seven probiotic use-associated cases of prolonged *B. clausii* bacteraemia (mean duration [range] 64 days [14–93 days] where data were available) in the literature, all with underlying co-morbidities.

Conclusion. *B. clausii* probiotic preparations may cause prolonged bacteraemia, rendering patients with underlying co-morbidities as well as those with unrecognized risk factors vulnerable for significant infectious complications.

INTRODUCTION

Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit to the host' [1]. The use of probiotic supplements in the USA is steadily rising – National Health Interview Surveys reported increased prebiotic and probiotic use in adults (0.4–1.6%) and children (0.3–0.5%) from 2007 to 2012 [2]. Common probiotic preparations contain bacteria (*Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Streptococcus*) and fungi (*Saccharomyces boulardii*) [1].

Bacillus clausii is a Gram-positive, aerobic, endospore-forming, facultatively alkaliphilic rod-shaped bacterium [3]. The spores germinate into vegetative forms that colonize the small intestine and can persist in faeces for up to 12 days after administration [4]. In fasting conditions, spore germination can occur rapidly, within 3 h of incubation, producing persistent bacterial growth [5]. The spores are heat stable and can survive in environments with poor nutrition, dehydration and low pH [6]. The *B. clausii*-containing probiotic Enterogermina (Sanofi-Aventis S.p.A.) was registered in 1958 and

has been available over-the-counter since 1999 [4]. In Asia, *B. clausii* probiotics have been used for acute diarrhoeal illnesses [7, 8]. However, there are rare reports of adverse effects due to probiotics [1].

We describe the prolonged persistence of *B. clausii* bacteraemia after probiotic use in an immunocompetent child.

CASE REPORT

A 17-month-old girl, born full term and with no significant past medical history, was admitted to our hospital for persistent fevers and positive blood cultures.

Prior to admission, she had travelled with her family to western India for 26 days in early 2020. While abroad, she was hospitalized for 2 days with non-bloody, non-bilious emesis and non-bloody diarrhoea. Testing revealed a negative malarial parasite smear and C-reactive protein (CRP) of 18 mg l⁻¹ [normal range (N): <5.0 mg l⁻¹]. She was discharged on oral cefixime and a probiotic containing *B. clausii* 2 billion spores per capsule (Enterogermina; Sanofi-Aventis S.p.A.),

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Keywords: *Bacillus clausii*; probiotic; bacteraemia.

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MRI, magnetic resonance imaging; MS, mass spectrometry; RVP, respiratory viral panel; SARS-CoV-2, Severe acute respiratory syndrome - coronavirus 2; USA, United States of America; WBC, white blood cell.

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Table 1. Antimicrobial sensitivity patterns in our patient and prior cases

Antimicrobial agent	MIC values ($\mu\text{g ml}^{-1}$) for antimicrobial sensitivity			
	CLSI guidelines [9]	17-month-old female (current case)	5-month-old male [10]	Middle-aged woman [13]
Ampicillin	S: ≤ 0.25 R: ≥ 0.5	0.38 (sensitivity could not be determined)	NA	NA
Ceftriaxone	S: ≤ 8 R: ≥ 64	8 (S)	NA	NA
Clindamycin	S: ≤ 0.5 R: ≥ 4	>256 (R)	NA	NA
Gentamicin	S: ≤ 4 R: ≥ 16	0.064 (S)	NA	NA
Penicillin	S: ≤ 0.12 R: ≥ 0.25	0.25 (R)	MIC-NA (S)	32 (R)
Ciprofloxacin	S: ≤ 1 R: ≥ 4	NA	NA	0.38 (S)
Levofloxacin	S: ≤ 2 R: ≥ 8	0.50 (S)	NA	NA
Rifampin	S: ≤ 1 R: ≥ 4	>32 (R)	NA	NA
Vancomycin	S: ≤ 4	0.50 (S)	MIC-NA (S)	0.50 (S)

CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration; MIC-NA, minimum inhibitory concentration data for the particular antibiotic not available; NA, not available; R, resistant; S, sensitive.

which she continued for 4 days. She had intermittent fevers (highest temperature 38.3 °C) for the remainder of her trip.

She presented to our paediatric emergency department 1 day after return to the USA for evaluation of persistent fevers; however, on arrival, she was afebrile and haemodynamically stable, with a normal physical exam. Laboratory testing revealed a white blood cell (WBC) count of 14510 μl^{-1} (N: 6000–17000 μl^{-1}), platelet count of 586000 μl^{-1} (N: 150000–400000 μl^{-1}), CRP of 10.1 mg l^{-1} and erythrocyte sedimentation rate (ESR) of 51 mm h^{-1} (N: 0–20 mm h^{-1}). Urinalysis was negative. Respiratory viral panel (RVP) testing of a nasopharyngeal swab revealed Coronavirus (non-SARS-CoV-2) infection. Blood cultures grew a Gram-positive bacillus after 2 days of incubation, so empiric ceftriaxone was initiated. The organism was identified as *B. clausii* by matrix-assisted laser desorption/ionization-time of flight MS (Beckman Coulter) and was confirmed by the state reference laboratory. She had 19 blood cultures growing *B. clausii* during her 31-day hospitalization.

Empiric ceftriaxone was switched to ampicillin, due to reports of ampicillin's efficacy against *B. clausii* [3]. Antimicrobial susceptibility was derived using Clinical and Laboratory Standards Institute guidelines – the isolate was susceptible to ceftriaxone, gentamicin, levofloxacin and vancomycin (Table 1) [9]. Persistent bacteraemia prompted addition of levofloxacin, with gentamicin added for synergy. Ampicillin was discontinued after further sensitivity testing was indeterminate and replaced with intravenous vancomycin, while continuing gentamicin

and levofloxacin. Oral vancomycin was added to target bacteria from germinating intraluminal spores.

She had an extensive evaluation for source and risk factors predisposing to persistent bacteraemia. Urine cultures revealed no growth. Repeat RVP testing was negative. Multiple transthoracic echocardiograms were negative for endocarditis. Abdominal ultrasound was unremarkable. Magnetic resonance imaging (MRI) of chest/abdomen/pelvis revealed no acute abnormalities, with patent arterial and venous vasculature. Brain MRI was unremarkable. Lower extremity ultrasound was negative for deep vein thrombosis. Positron emission tomography scanning did not reveal a source of infection. Vitamin A and zinc levels were normal; however, zinc/multivitamin supplementation was started to prevent micronutrient deficiency and bacterial translocation.

Immunology was consulted to rule out underlying immunodeficiency. Evaluation revealed normal quantitative immunoglobulins and protective titres to tetanus, diphtheria, measles, mumps, rubella and *Streptococcus pneumoniae* (consistent with normal humoral immunity). Assessment of cellular immunity revealed a normal lymphocyte immunophenotype (normal CD4+ and CD8+ T cells and CD19+ B cells) and normal lymphocyte function (based on phytohaemagglutinin/pokeweed mitogen response). Complement assays including total complement (CH50), alternate pathway (AH50) and mannose binding lectin were normal. Phagocytic evaluation revealed normal dihydrorhodamine (DHR), glucose-6-phosphate-dehydrogenase and myeloperoxidase staining.

Table 2. Reports of *Bacillus clausii* bacteraemia in the literature (including current case)

	Age/Sex	Past medical history	Symptoms	Antibiotics	Duration of bacteraemia	Outcome	Reference
Pt #1	17 months/Female	None	Fevers, elevated WBC count	Ceftriaxone (IV), ampicillin (IV), gentamicin (IV), levofloxacin (IV), vancomycin (IV), vancomycin (PO), levofloxacin (PO)	111 days	Recovery	Current case
Pt #2	5 months/Male	Surgically corrected congenital heart disease	Fever, respiratory distress	Vancomycin (IV)	Approximately 58 days*	Death (<i>B. clausii</i> sepsis recurrence and <i>K. pneumoniae</i> sepsis)	[10]
Pt #3	NA	Stage 4 lung cancer	Fever, elevated WBC count	NA	NA	NA	[11]
Pt #4	NA	Stage 4 lung cancer	Fever, elevated WBC count	NA	NA	NA	[11]
Pt #5	NA	Ischaemic colitis	Fever, elevated WBC count	NA	NA	NA	[11]
Pt #6	69/Male	Aortic valve replacement	Fevers	Vancomycin, rifampin, levofloxacin, tigecycline (IV)	70 days	Recovery	[12]
Pt #7	71/Male	Mitral valve replacement, tricuspid valve replacement	Fever	Vancomycin (IV), levofloxacin (IV)	93 days	Recovery	[12]
Pt #8	Middle aged/ Female	Diabetes mellitus type-2	Fever	Teicoplanin	14 days	Recovery	[13]

F, female; IV, intravenous; M, male; NA, not available; PO, oral; WBC, white blood cell.

*An approximate duration of 58 days was derived after review of the case report. The patient had received probiotics at 3 months of age and he presented with sepsis 3 weeks later. He again presented for surgery at 5 months of age and was found to have bacteraemia recurrence, lasting at least 3 weeks.

Toll-like receptor testing was not performed due to laboratory error. A genetic panel of 207 primary immunodeficiency genes revealed heterozygous variants of uncertain significance: CHD7 c.5066T>C (p.Val1689Ala), RMRP n.228C>T (RNA change), RTEL1 c.1189C>G (p.Gln397Glu), which were not consistent with her phenotype.

She was discharged on oral vancomycin and levofloxacin to complete a 14-day course. During outpatient follow-up, she remained clinically stable without antibiotics. *B. clausii* grew in surveillance blood cultures drawn 1 week, 5 weeks and 11 weeks from hospital discharge. Her CRP levels remained undetectable, with minimally elevated ESR (22 mm h⁻¹) and unremarkable haematological, renal and hepatic laboratory parameters. By 12 and 16 weeks after hospital discharge, blood cultures were sterile.

We performed a comprehensive literature review in the Medline and Google Scholar bibliographic databases for English language articles with the search terms '*Bacillus clausii* bacteraemia' and 'probiotics bacteraemia'. We identified seven prior cases of

B. clausii bacteraemia occurring in patients with underlying co-morbidities (Table 2) [10–13].

DISCUSSION

B. clausii bacteraemia has been previously described in only a few patients with underlying illnesses [10–13]. A 5-month-old child with congenital heart disease and malnutrition developed recurrent *B. clausii* bacteraemia (persisting for more than 10 days in each instance) and subsequently succumbing to multidrug-resistant *Klebsiella pneumoniae* sepsis with multi-organ failure [10]. *B. clausii* bacteraemia was also noted in three adults with advanced lung cancer and ischaemic colitis [11]. Prolonged bacteraemia for 70 and 93 days respectively was reported in two adults after valve replacement, despite antimicrobial therapy [12]. Lastly, an adult diabetic patient, who had received antibiotics and probiotics for a surgical-site infection, developed *B. clausii* bacteraemia that resolved after 2 weeks with teicoplanin [13].

To the best of our knowledge, this is the first case of *B. clausii* bacteraemia occurring in an immunocompetent paediatric patient. The reason for bacteraemia lasting more than 100 days, while on combined antimicrobial therapy, is unknown. The evaluation did not reveal any previously undiagnosed co-morbidity, as seen in previously reported cases [10–13]. Our suspicion for an intravascular source or immunocompromised state was low, given negative comprehensive workup, haemodynamic stability and rapid symptom resolution.

In some cases, bacteraemia following probiotic use is postulated to occur secondary to intestinal bacterial translocation, particularly in the setting of pre-existing intestinal pathology (such as diarrhoea or anatomical gut alterations) [1]. In our case we hypothesize that a persistent intra-luminal focus of *B. clausii* spores germinated over time and allowed prolonged or recurrent bacterial translocation through the bowel wall, which was facilitated by the antecedent diarrhoea illness. The median (range) duration of bacteraemia noted in the reviewed cases (including our case) was 70 days (14–111 days) (Table 2), supporting our hypothesis of an intra-luminal focus of germinating *B. clausii* spores with intermittent bacterial translocation. Such transmigration can occur regardless of a patient's immune status [13].

Probiotic organisms have antimicrobial resistance genes, which facilitate their concurrent administration with antibiotics – thus, treating infections caused by probiotic organisms is challenging [14, 15]. The chromosomally located, non-transferable *erm* gene in *B. clausii* produces a macrolide–lincosamide–streptogramin-B phenotype, which results in macrolide resistance [16]. Additionally, *B. clausii* has been shown to possess intrinsic resistance to penicillins, cephalosporins, aminoglycosides and macrolides [17]. Marketed in India for use in paediatric diarrhoeal illness, the probiotic used by our patient contains four different *B. clausii* strains [18]. The strains are named according to their antibiotic resistance: ‘O/C’ resistant to chloramphenicol; ‘N/R’ resistant to novobiocin and rifampin; ‘T’ resistant to tetracycline; and ‘SIN’ resistant to neomycin and streptomycin [18]. All four strains were sensitive to multiple antibiotics (including penicillins, glycopeptides, quinupristin, dalfopristin, linezolid and fluoroquinolones) and resistant to cefuroxime, ceftriaxone, cefotaxime and cefepime [3]. In our patient, *B. clausii* was resistant to penicillin, rifampin and clindamycin, while sensitivity to ampicillin could not be determined. Antimicrobial resistance was also noted in some of the prior reported cases (Table 1).

There are mixed data available regarding the efficacy of *B. clausii* probiotics. A systematic review of six trials showed that *B. clausii* helped decrease hospitalization duration by 1 day [7]. Another multi-centre study found that *B. clausii* compared to zinc administration helped to decrease diarrhoea duration and mean daily stool output; however, the group receiving zinc had worsening diarrhoea (compared to no intervention), making interpretation of these results unclear [8]. In pre-term infants, *B. clausii* failed to show a significant effect on incidence of late-onset sepsis [19].

Until now, the safe administration of probiotics has focused on potentially avoiding them in patients with compromised immune systems or underlying serious illnesses [1, 15]. In one review, it has been proposed to give probiotics cautiously in patients with a single major risk factor (immunocompromised status, prematurity), or with more than one minor risk factor (presence of central venous catheter, probiotic administration through jejunostomy, underlying heart anomaly, disrupted intestinal epithelial integrity, probiotics with high mucosal adhesion/known pathogenicity or use of broad-spectrum, probiotic-resistant antibiotics) [1]. In contrast, our case highlights the concerning finding that probiotics can cause bacteraemia in healthy individuals. How often this occurs and its related clinical consequences are not clear. The paucity of reporting so far could be either due to the bacterium's low pathogenicity, or to the tendency to regard *Bacillus* species as blood culture contaminants [20]. Given the ever-increasing worldwide probiotic usage, clinicians should be vigilant about the concerning potential of *B. clausii*-containing probiotics to cause prolonged bacteraemia, even in immunocompetent hosts. Additional investigations are needed to evaluate risk factors and help elucidate mechanisms that lead to gastrointestinal translocation of these bacteria.

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Author contributions

A.M.K., S.H.F.H. and M.K.K. were involved in conceptualization, writing of the original draft, and review and editing of subsequent drafts. S.H.F.H. and M.K.K. were also involved in supervision. S.R. and C.S. were involved in writing of the original draft and review and editing of subsequent drafts. A.M. and B.K. were involved in review and editing of subsequent drafts. All authors reviewed the final manuscript prior to submission.

Conflicts of interest

The authors declare that there are no conflicts of interest

Ethical statement

The research met our institutional definition of a case report (a medical chart review of three or fewer patients), and thus institutional research board review was not needed. The parents of our patient reviewed the manuscript prior to submission. Informed consent was obtained from the father of the patient.

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