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Case Report

Bifidobacterium bacteraemia is rare with routine probiotics use in preterm infants: A further case report with literature review

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ABSTRACT

Prophylactic administration of oral probiotics is associated with significant reductions in the morbidity and mortality of necrotising enterocolitis in preterm infants. We document the first case of *Bifidobacterium longum* subsp. *infantis* sub-clinical bacteraemia, in an extremely low birth weight preterm infant, since introduction of routine probiotic treatment at the Norfolk and Norwich University Hospital 10 years ago. Whole genome comparisons confirmed the isolated strain likely originated from the probiotic product.

1. Introduction

Neonates, and preterm infants in particular, often develop life-threatening conditions due to an immature gut microbiota and immune system [1]. Necrotising enterocolitis (NEC), the most frequent gastrointestinal emergency in preterm infants, is a multi-factorial condition associated with overgrowth of potentially pathogenic microbiota members, and may result in intestinal perforation, and abdominal cavity infection [2]. In recent years, oral probiotics are estimated to be used in 17% of tertiary-level Neonate Intensive Care Units (NICUs) in England, according to a 2018 survey [3], to alter gut microbiota profiles beneficially, and to improve preterm health outcomes reducing NEC-associated morbidity and mortality by $\geq$ 50% [4,5]. *Bifidobacterium* species and strains are included in many currently available probiotic formulations owing to their long-standing safety track record, ability to breakdown specific dietary components (e.g. human milk oligosaccharides), and their anti-inflammatory and immunomodulatory properties [6]. Although classed as 'generally recognised as safe', there are concerns of potential *Bifidobacterium* probiotic-associated bacteraemia and/or sepsis in at-risk infants, however there are only a few documented cases to date [7,8].

Here, we report a further case of non-fatal *Bifidobacterium* bacteraemia associated with probiotic treatment in an extremely low birth weight infant. *B. longum* subsp. *infantis* was isolated from a blood culture and, using comparative genomics, we confirmed that the isolate recovered from the infant originated from the probiotic formulation.

2. Description of the case

A female infant weighing 490 g was delivered at 24 weeks and 4 days' gestation by lower segment Caesarean section. She was small for gestational age (birth weight <10th percentile). She was admitted to NICU, required intubation and ventilation, and developed pneumothorax and pneumatoceles. The admission blood culture was negative. Umbilical venous and arterial catheters were sited on the first postnatal day of life (DOL 1) and intravenous parenteral nutrition feeds commenced. On DOL 11 the umbilical arterial catheter was removed and on DOL 15 the umbilical venous catheter was replaced by a peripherally-inserted central venous catheter (Fig. 1). Enteral feeding with maternal colostrum commenced on DOL 2 but were stopped the same day due to

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periodic bilious aspirates, and maternal colostrum/breastmilk was only restarted on DOL 13 when the bilious aspirates cleared. The infant received a first dose of probiotics on DOL 2 and supplementation continued daily despite enteral feeds being withheld. Multi-species oral probiotics (*Lactobacillus* and *Bifidobacterium* spp.) have been routinely used in our NICU for prophylaxis of NEC since January 2013. Since 2016, we have used the commercial product Labinic Drops™ (Biofloratech, UK). The daily dosage of Labinic Drops given to the infant (5 drops; ~0.2 mL) provided ~2 billion colony forming units of live bacteria (*Lactobacillus acidophilus* 0.67 × 10^9, *Bifidobacterium bifidum* 0.67 × 10^9, and *Bifidobacterium longum* subsp. *infantis* 0.67 × 10^9). On DOL 15 an unexpected increase in C-reactive protein (CRP), from 7 to 52 mg/L, was noted in the routine daily blood panel. This prompted an infection screen and empirical commencement of antibiotics (Fig. 1). Concomitant complete blood count revealed a high white blood cell count, 35 × 10^9/L (neutrophils 25.8 × 10^9/L), and low platelets 112 × 10^9/L. Manual blood film examination showed neutrophil leucocytosis and neutrophils showed a left shift. She had no overt clinical signs or symptoms of infection at this time. Her peripheral blood culture isolated *Bifidobacterium* spp. after 2 days' incubation (BacT/ALERT® PF Plus (PF Plus), bioMérieux Inc., USA). She remained stable over the next 3 days and CRP fell to 32 then 22 mg/L. However, on DOL 18, she developed acute abdominal distension and increased ventilatory requirements. Abdominal perforation was suspected, enteral feeds, probiotics, and dexamethasone were stopped, and abdominal x-ray confirmed pneumoperitoneum. CRP rose again to 52 mg/L. A diagnosis of spontaneous ileal perforation was made at laparotomy on DOL 19, at which an ileostomy was formed. Surgical histopathology excluded NEC and was consistent with isolated spontaneous intestinal perforation (SIP). A repeat blood culture taken preoperatively (DOL 18) grew *Staphylococcus epidermidis* after 1 day incubation but was negative for *Bifidobacterium* spp. Piperacillin-tazobactam (90 mg/kg 8 hourly) was substituted for Cefotaxime, and given for 5 days, and vancomycin continued for 11 days. CRP peaked at 95 mg/L on DOL 19. Probiotic treatment resumed on DOL 23, and enteral feeding on DOL 25 (Fig. 1). The infant was discharged home 7 months after birth weighing 4.2 Kg; at discharge she required no supplementary oxygen, was being fed via nasogastric tube, and still had her stoma *in situ*.

The *Bifidobacterium* spp. isolate recovered from the infant's blood culture (DOL 15) was retrieved from the clinical diagnostic laboratory and cultured on de Man-Rogosa-Sharpe (MRS) (Oxoid) medium supplemented with 0.5 g/L cysteine-HCL for 48h under anaerobic conditions (A20 workstation, Don Whitley Scientific, UK), and subjected to whole genome sequencing (WGS) (Illumina Nextseq500) in our laboratory. Independently, the content of the Labinc Drops ™ was inoculated on MRS agar and incubated anaerobically as above, with the resulting isolates subjected to WGS (Illumina HiSeq 2500) at the Wellcome Trust Sanger Institute (Hinxton, UK). Additionally, the publicly-available sequence for the Danisco Florafit *B. longum* subsp. *infantis* Bi-26 contained in the Labinic Drops ™ (accession number: CP054425.1) was retrieved from NCBI Genome database [9].The genomes of *Bifidobacterium* isolates recovered from both the infant's blood culture and the Labinic Drops ™ were compared with that of *B. longum* subsp. *infantis* Bi-26 using the average nucleotide identity (ANI) algorithm [10] and single nucleotide polymorphism (SNP) variant calling [11]. This analysis revealed the ANI score above 99.9% and the SNP distance of less than 10 SNPs between the three genomes, leading to the firm conclusion that the isolate in infant's blood originated from the commercial probiotic product used in our NICU (Fig. 2).

For further corroboration, the genomes were screened for the presence of antimicrobial resistance genes against both an in-house sequence collection and the CARD database [12], which revealed the presence of putative homologues associated with conferring of resistance to tetracycline (*tet(M)/tet(W)/tet(O)/tet(S)*) [13] and rifampicin (*rpoB*) [14], (Fig. 3). These findings were in line with previous reports for bifidobacteria showing very limited antibiotic resistance profiles, including for those strains used in probiotics [15,16].

3. Discussion

This report describes a rare case of probiotic-associated bacteraemia in a routinely probiotic-supplemented extremely low birth weight preterm infant whose initial clinical manifestation comprised only abnormal laboratory markers. The bacteraemia was rapidly cleared with a standard antibiotic combination. WGS and

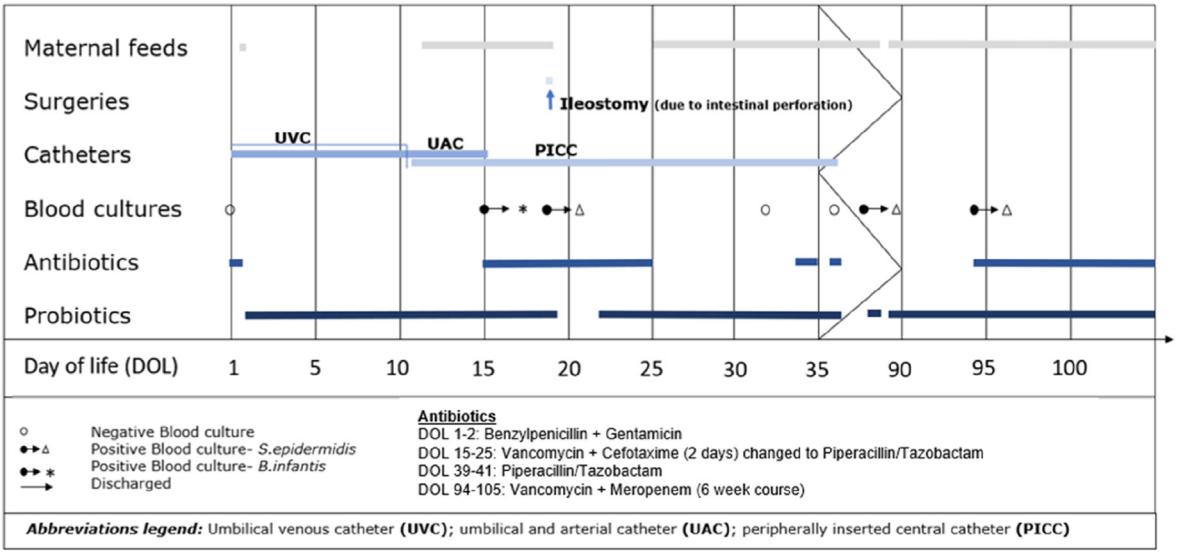

Fig. 1. Timeline of the infant's bacteraemia episodes, diagnostic, and treatment pathway. Umbilical venous catheter (UCV), umbilical arterial catheter (UAC), and peripherally-inserted central venous catheter (PICC). Surgery details; DOL 18 spontaneous intestinal perforation (SIP), and DOL 19 laparotomy (+ileostomy).

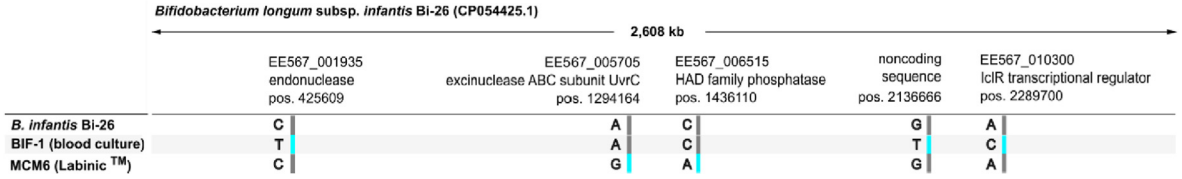

Fig. 2. Graphical representation of SNP distribution over *B. longum* subsp. *infantis* genomes.

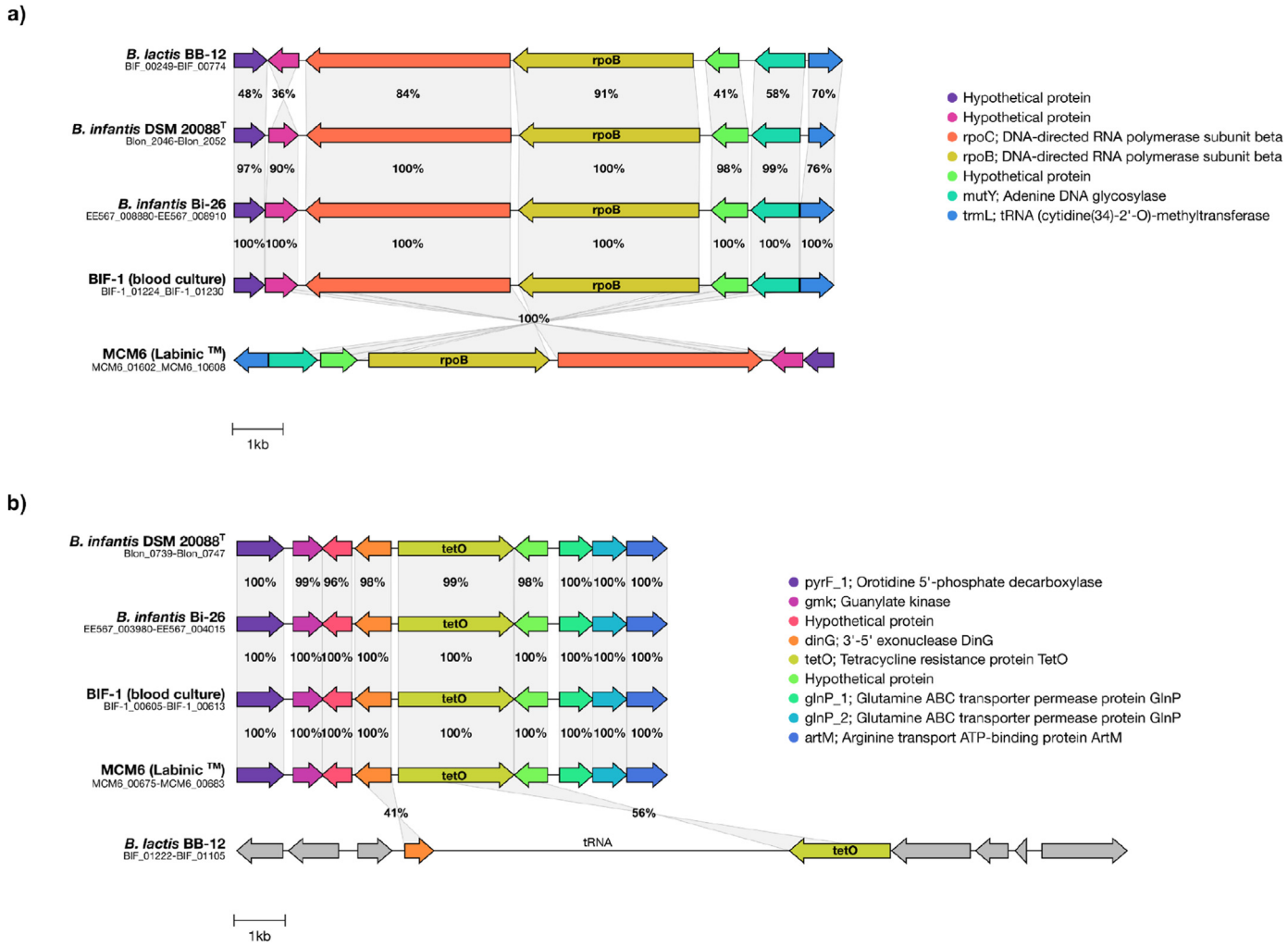

Fig. 3. Representation of the chromosomal regions flanking putative genes associated with conferring resistance to **(a)** rifampicin (rpoB) and **(b)** tetracycline (tetO) in selected *Bifidobacterium* strains, including isolates from this report and known probiotics (*B. infantis* Bi-26 and B. *lactis* BB-12). Genes with amino acid identity over 30% are represented with the same colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analysis confirmed the blood isolate to be the identical strain present in the given commercial probiotic supplement (Labinic Drops ™). In the last 10 years, to the best of our knowledge, only three previous case reports of *B. longum* subsp. *infantis* sepsis or bacteraemia have been published, comprising a total of six very low birth weight (<1500 g) preterm infants, all of whom also fully recovered (Table 1). In 5 of the 6 cases, infants had suffered from NEC or SIP, with necrosis, inflammation or surgical intervention suggested as the primary causes of bacterial translocation [17,18]. Histopathological difference between NEC and SIP can be unclear, which may lead to SIP and NEC misclassification [19,20].

While the infant presented in this case report also suffered temporally-proximate bowel perforation requiring surgery, we have shown that the raised CRP and *Bifidobacterium*-positive blood culture prefaced the perforation by 3 days. The development of local oedema and inflammation in the infant's ileum could have allowed translocation of *Bifidobacterium* from the gut, as these exact features were observed histopathologically in the resected gut segment. We estimate that globally to date, hundreds of thousands of preterm infants have now received multiple doses of probiotics prophylactically during their NICU stays, and yet reports of probiotic bacterial sepsis/bacteraemia are extremely rare. This highlights the exceptionally strong safety record of probiotics as an effective NEC treatment, now including our own experience with only this single case of sub-clinical bacteraemia among >1000 infants treated with *Bifidobacterium-Lactobacillus* combination

Table 1
Description of 6 different *B. longum* subsp. *infantis* related sepsis and bacteraemia cases in preterm infants within the last 10 years.

Reference	Etiologic agent	Infection type	Gestational age, sex Weigh, birth method	Underlying conditions	Probiotic treatment (days)	Antibiotic Treatment (days)	Outcome
[21]	*B. longum* subsp. *infantis*	Sepsis, Bacteraemia,	(1) 24, Male,730 g, vaginal	(1) sepsis, pneumoperitoneum, features of NEC	*(1,)* 8	(1, 2, 3) Not specified	(1) Recovered
		Sepsis	(2) 23, Male,500 g, vaginal	(2) apnea, bradycardia, and temperature	(2) 12		(2) Recovered
			(3) 24, Female,697 g, C,-section	(3) NEC, Bowel perforations, ventilation	(3) 46		(3) Recovered
[22]	*B. longum* subsp. *infantis*	Sepsis,	(1) 26, Female, 867 g, vaginal	(1) tachycardia, ileus, intestinal distention, anastomosis, intestinal necrosis	(1) 14	(1) 7, Ceftazidime & Vancomycin; 7, Imipenem	(1) Recovered
		Bacteraemia	(2) 28, female, 1090 g, C-section,	(2) nasal O2, abdominal distention, intubation, transfusion, leukopenia, pneumatosis intestinalis, NEC, necrosis, jejunal perforation, reinsertion of small intestine, anastomosis	(2) 10	(2) 3, Amoxicillin and Gentamicin; N/A, Ceftazidime, Amikacin, and Metronidazole	(2) Recovered
[23]	*B. longum* subsp. *infantis*	Bacteraemia	28, Female, 1090 g, C-section	Abdomen distension, coagulopathy (NEC)	4	(not specified), Ceftazidime, Amikacin, Metronidazole	Recovered

probiotics in our centre over the past decade, and despite individual infants typically receiving daily doses of probiotics for a duration of at least 30–60 days depending upon birth gestation. Many studies have shown that *Bifidobacterium* is a beneficial member of the early life gut microbiota, and its presence is associated with numerous health benefits including strengthening of the neonatal gut barrier and induction of homeostatic and anti-inflammatory immune responses [24–27]. This contrasts with the typical pro-inflammatory cascade associated with non-probiotic species bacterial sepsis. Moreover, regulations for probiotics state that the strains used must not have acquired antibiotic resistance. The *B. longum* subsp. *infantis* contained within the Labinic formulation fulfils this requirement, which links to its rapid elimination from subsequent blood cultures after second-line antibiotics.

We have documented a rare case of non-fatal probiotic-associated bacteraemia caused by a *B. longum* subsp. *infantis* in an extremely low birth weight preterm infant. It was isolated from blood cultures taken due to a raised CRP but no clinical signs, and genomic approaches confirmed the probiotic provenance of the bifidobacterial strain detected in the infant's blood. The portal of its entry into the bloodstream was most likely translocation at the site of evolving gut pathology.

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CRediT authorship contribution statement

Antia Acuna-Gonzalez: Investigation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Magdalena Kujawska:** Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Mayada Youssif:** Investigation, Formal analysis, Writing – review & editing. **Thomas Atkinson:** Investigation, Writing – review & editing. **Sara Grundy:** Investigation, Methodology, Resources, Writing – review & editing. **Alexandra Hutchison:** Methodology, Writing – review & editing. **Catherine Tremlett:** Investigation, Methodology, Resources, Writing – review & editing. **Paul Clarke:** Investigation, Formal analysis, Resources, Supervision, Conceptualization, Project administration, Writing – original draft, Writing – review & editing. **Lindsay J. Hall:** Formal analysis, Resources, Supervision, Funding acquisition, Conceptualization, Investigation, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest relevant to this article.

Data availability

Genomic sequence data is available via project number; PRJNA882778

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