

Clinical and nutritional management in subjects with different intestinal failure types: characterization of intestinal bacterial communities in a case series

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Abstract

Introduction

Short Bowel Syndrome (SBS) is a complex paediatric condition and the primary pathophysiological cause of intestinal failure (IF), resulting from a significant reduction in the length of the small intestine. This reduction in absorptive surface area leads to nutrient malabsorption, impaired digestion, and other clinical complications.

Case series

This case series explores the clinical aspects of seven paediatric SBS subjects grouped according to IF classification associated with the variation in the intestinal microbiota composition. Blood and urine samples were collected twice a week during hospitalization to evaluate clinical parameters. Faecal samples collected before and after intestinal surgical intervention were analyzed for microbiota composition using a targeted metagenomics sequencing approach, focusing on the V3-V4 region of the 16S rRNA gene on the MiSeq (Illumina) platform.

Results

We observed specific variations in blood and urinary analytes according to the IF category as well as variations in the diversity and composition of the intestinal bacteria communities. Particularly, variations in the diversity were associated with specific changes in 15 bacterial ASVs abundances among the variables considered (*i.e.* IF category, residual colon anatomy and specific clinical history (patient variable)).

Conclusions

This study provides valuable insights into the clinical history of paediatric cohorts with SBS, expanding the knowledge base for their clinical and nutritional management. Characterizing the intestinal microbiota in these patients plays a crucial role in identifying potential markers of dysbiosis which may provide important insights for developing targeted pharmacological therapies and personalized care for SBS patients. Monitoring specific microorganisms could enable timely pharmacological or clinical intervention, leading to personalized therapies (*e.g.*, targeted antibiotic therapy). This case series highlights the complexity of SBS and the necessity of an integrated approach, in which microbiota composition can be leveraged to address dysbiosis, ultimately improving the quality of life of paediatric patients.

INTRODUCTION

Short bowel syndrome (SBS) is a complex disorder characterized by a marked reduction in the functional length of the small intestine, resulting in intestinal failure (IF). With an estimated incidence of 25 per 100,000 live births, SBS most commonly arises from congenital anomalies, extensive surgical resections

[1, 2], or severe intestinal diseases, and is associated with a wide spectrum of clinical manifestations that profoundly affect quality of life, particularly in paediatric populations [3, 4]. Patients with SBS frequently experience malnutrition, dehydration, and electrolyte imbalances, especially following extensive colonic resection [5].

Reduction of the intestinal absorptive surface results in malabsorption of macro- and micronutrients, with the severity depending on the extent of bowel resection. Consequently, these patients require parenteral nutrition for survival; however, long-term parenteral nutrition is associated with severe complications, including catheter-related sepsis and metabolic disorders. Spontaneous intestinal adaptation occurs within weeks to months after resection and is characterized by alterations in gut hormone levels, hyperplasia of the remnant intestine, intestinal dysbiosis, and hyperphagia [6]. Indeed, impaired secretion of key regulators of gut motility, contributes to increased disease severity and clinical complexity. Conversely, enterohormones implicated in the ileal brake mechanism, such as GLP-2, GLP-1, and PYY, are essential in driving intestinal adaptation and may promote accelerated or hyperadaptive responses [7].

Increasing attention has recently focused on the role of the intestinal microbiota in SBS. The composition and structure of the gut microbiota vary along the gastrointestinal (GI) tract and play a fundamental role in maintaining systemic homeostasis and essential physiological functions [8, 9,10]. The gut microbiota contributes to immune system education by supporting immune recognition and defence against external pathogens, while also promoting intestinal mucosal growth and barrier integrity [11, 12]. In SBS patients, microbial dysbiosis may compromise the intestinal barrier, triggering inflammation and contributing to multiple clinical complications [13,14]. Moreover, the anatomic and physiologic changes associated with short bowel syndrome (SBS), together with medications commonly used in these patients, facilitate the development of small intestinal bacterial overgrowth, which is further promoted by impaired intestinal motility and other predisposing factors and frequently results in symptoms such as gas, bloating, abdominal cramping, and diarrhea [15].

This study aims to describe a paediatric case series of SBS patients stratified according to intestinal failure (IF) classification. We provide a comprehensive clinical characterization, with particular emphasis on changes in blood and urinary analytes before and after intestinal remodelling surgery. To further enhance case series characterization, we also analysed the intestinal microbiota, highlighting differences associated with IF classification. Specifically, we sought to identify changes in microbial diversity and bacterial signatures linked to distinct IF categories. This investigation contributes to the identification of dysbiosis biomarkers and underscores the critical role of gut microbiota analysis as a potential tool for guiding personalised therapeutic strategies in SBS.

CASE SERIES

The study was conducted from January 2018 to January 2024, encompassing a total duration of 6 years at the Department of Paediatric and Neonatal Surgery, Meyer Children's Hospital IRCCS, Florence, Italy.

The Ethics Committee of the Meyer Children's Hospital IRCCS granted ethical clearance for this study (protocol number GDF15).

Patient enrollment took place over a period of 24 months. A strict restriction was imposed on the use of specific medications, including laxatives, antidiarrheals, anti-inflammatory agents, corticosteroids, proton pump inhibitors, and transit modulators for a duration extending up to six weeks before baseline sample collection. These patients referred to our institution for a surgical evaluation were classified based on the type of IF determined by the anatomy of the remaining bowel according to the criteria proposed by Pironi [16] (Type 1, end-jejunostomy/ileostomy; Type 2, jejunocolonic anastomosis, where the remaining jejunum is connected to a portion of the left colon; Type 3, jejunoleocolonic anastomosis with the ileocecal valve and colon in continuity). Furthermore, according to the ASPEN 2022 criteria, paediatric IF has been defined as the reduction in functional intestinal mass leading to a level insufficient to sustain life, requiring supplemental parenteral nutrition for at least 60 days within a consecutive 74-day period [17]. In these patients, the residual bowel length should be measured along the antimesenteric border from the duodenojejunal flexure to the ileocecal valve or to the small bowel-colon anastomosis. In addition, a contrast study was performed to better characterize the intestinal anatomy, including residual small bowel length, bowel dilatation, and the presence of the colon [18].

At the beginning of the study, a pediatric surgeon specialized in SBS management conducted an interview with each participant to document their symptoms in detail. Importantly, at the time of recruitment, none of the subjects presented with organic pathologies or IF secondary to hepatic dysfunction. Before inclusion in the study, all participants or their guardians were fully informed and provided written informed consent. The participants' families were instructed to carefully record a numerical rating scale (NRS) assessing symptoms such as abdominal pain, discomfort, bloating, sensation of incomplete evacuation, defecatory straining, and overall dissatisfaction with bowel movements. This evaluation ranged from 1 (indicating minimal discomfort) to 7 (indicating maximum discomfort). Additionally, the Bristol Stool Form Scale, ranging from 1 (indicating lumpy stools) to 7 (indicating watery stools), along with the daily frequency of bowel movements, was recorded over a two-week period starting from study initiation. Patients and controls were assessed within this timeframe, with sample collection conducted before the surgical procedure and at three intervals throughout the year. To ensure consistency and reliability in data analysis, samples were collected in triplicate. Venous blood samples were obtained, and comprehensive anthropometric evaluations were performed on all participants within the study framework. At the time of sample collection, all patients were on a parenteral nutrition (PN) regimen to supplement their oral intake. Details of PN volumes, infusion hours, and frequency per week are provided in the case presentations. An overview of nutrients administered through parenteral nutrition were resumed in Figure S1. PN composition was carefully adjusted during hospitalization based on serial blood tests, performed twice weekly (on Mondays and Tuesdays), to monitor for electrolyte imbalances, protein levels, and signs of dehydration. PN was supplemented with fat-soluble and water-soluble vitamins as needed, and lipids were administered using SMOFlipid formulations. In cases where hepatic function preservation was necessary, lipid administration was omitted on certain nights of the week. PN was delivered via cyclic overnight infusions, with a temporary

shift to continuous infusion in the days immediately following surgery. The cyclic regimen was reinstated as soon as enteral nutrition became feasible. All the patients followed the Short Bowel Protocol established at our center that includes routine blood and urine tests conducted every Monday and Tuesday to monitor electrolyte balance, hemoglobin levels, hepatic, pancreatic, and renal function, vitamin absorption, coagulation status, and urinary electrolytes. Additional tests were performed as needed based on the patient's clinical condition and evolution.

The present investigation encompassed a cohort of seven paediatric subjects diagnosed with SBS, with an age distribution characterised by a mean and standard deviation of 11.18 ± 8.51 years. The cohort included two females and five males (cohort's characteristics are resumed in Table 1). Patients were assigned according to the IF classification [16, 17], and stratified according to the percentage of residual colon anatomy (approximate percentage of 0, 25, 75, 100%). The patient's characteristics are comprehensively resumed in Table 2.

Case 1

Patient CZ is a male born at term with a prenatal diagnosis of multicystic kidney disease and intrauterine growth restriction (IUGR). He was delivered via cesarean section with a birth weight of 2880 g. Shortly after birth, he developed abdominal distension, followed by bilious vomiting and delayed passage of meconium. He subsequently experienced fecaloid vomiting and underwent his first surgery at 14 days of life, during which an ileostomy was created 80 cm from the Treitz angle. Histopathological analysis of biopsies taken 15 cm distal to the Treitz angle revealed the absence of ganglion cells, a finding confirmed upon review by two independent pathologists. He was diagnosed with very long-segment Hirschsprung disease.

At 2 months of age, the patient experienced intestinal obstruction, necessitating a revision of the ileostomy, which was relocated to 20 cm from the Treitz angle. Further revisions were required due to prolapse at 3 months of age and dehiscence at 9 years of age.

At 17 years old, the patient presented to our care, reporting a poor quality of life primarily due to the presence of a jejunostomy and ileostomy, as well as significant peristomal skin inflammation. He had a port-a-cath central line, placed after multiple Broviac infections caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, and *Candida albicans*. His nutritional support included PN seven days per week, administered over 10 hours nightly at a maximum rate of 240 mL/h. The formula was supplemented with Vitalipid and Soluvit three nights per week, while lipid-free formulations were used on two nights. He was not receiving intestinal decontamination therapy. The patient reported significant variability in stomal output related to oral intake, particularly after consuming dairy products.

At 17 years of age, the patient underwent bowel assessment and surgical remodeling. The initial procedure involved the creation of a new jejunostomy and a Ziegler myotomy in the distal intestine due

to a marked difference in caliber between intestinal segments. The distal stoma was noted to be stenotic, while the proximal intestine appeared dilated, measuring 6 cm in diameter and 25 cm in length.

Four months later, the patient was readmitted for a second surgical intervention which involved a total colectomy with ileocecal valve resection and removal of the aganglionic segment of the distal ileum. Prior to surgery, the patient was not receiving any antibiotic therapy. Postoperatively, he was started on a regimen of metronidazole and ceftazidime three times per day, along with gentamicin once daily. Gentamicin was discontinued after one week of treatment, while metronidazole and ceftazidime were continued for 10 days before being transitioned to oral amoxicillin which was maintained until discharge. At follow-up two years after surgery, the patient is in good clinical conditions, with reduced stomal output and no skin inflammation. He is currently on the waiting-list for a stoma closure procedure.

Case 2

Patient NF is a female diagnosed with SBS secondary to a midgut volvulus that occurred shortly after birth, leading to multiple surgical interventions due to complications related to her condition. Following the initial surgery, which involved volvulus derotation and abdominal closure, she experienced intestinal perforation, requiring a second surgery. During this procedure, the entire jejunum was resected, and a duodenal-ileal anastomosis was performed.

Subsequent complications, including dehiscence of the anastomosis and peritonitis, necessitated further interventions. These included total ileum resection, creation of a duodeno-colic anastomosis, and a colostomy formation. Five months later, the colostomy was closed.

At the age of five, the patient underwent bowel assessment and remodeling surgery, which included colonic interposition, with 10 cm of the sigmoid colon interposed between the duodenum and the colon. During this procedure, it was noted that she had 5 cm of ileum and 75% of her native colon remaining, with preservation of the ileocecal valve.

Prior to the remodeling surgery, the patient was on a prophylactic intestinal decontamination regimen alternating weekly between oral rifaximin and oral metronidazole. At the time of surgery, she was being treated with oral rifaximin. At the time of surgery, she was receiving oral rifaximin. Postoperatively, she was started on a regimen of metronidazole and ceftazidime three times per day, along with gentamicin once daily.

Before bowel assessment and remodeling surgery, the patient was dependent on PN seven nights per week, infused over 14 hours per night, with a total volume of 1.5 litres. She was also receiving oral nutrition. At that time, she measured 100 cm in height and weighed 14.5 kg, both below the 5th percentile for her age and sex.

At her last follow-up, three years post-surgery, she was receiving PN five nights per week, supplemented with lipids and Vitalipid nightly and Soluvit three nights per week. PN was administered over 11 hours per

night, with a total volume of 1.2 litres. She reported 4–6 bowel movements per day. Her height was 127 cm, placing her at the 10th percentile, while her weight was 22 kg, remaining below the 5th percentile for her age.

Case 3

Patient RS is a male born at term, diagnosed shortly after birth with type IIIA colonic atresia, presenting with abdominal distension, failure to pass meconium, and vomiting. He underwent urgent surgery involving colonic resection and stoma creation. On the seventh day of life, he developed signs of necrotizing enterocolitis, necessitating additional surgery during which 75 cm of ileum and 5 cm of colon were resected. At 32 days of life, stoma closure was performed along with the creation of an ileocolic anastomosis. At two years of age, the patient underwent the Serial Transverse Enteroplasty (STEP) procedure, which identified 45 cm of remaining ileum. The following year, he required another surgery involving stricturoplasty and possible additional intestinal resection, resulting in an estimated 35 cm of small intestine. At age four, he was diagnosed with stenosis at the ileocolic anastomosis, which was surgically treated by resecting the stenotic segment and creating a new anastomosis. The patient was referred to our center for bowel assessment and remodeling at six years of age. At the time of surgery, he had 55 cm of remaining small intestine, no ileocecal valve, and 25% of his native colon (approximately 45 cm). His weight was 16 kg and his height was 109 cm, both below the 5th percentile for age. He was dependent on PN seven nights per week, infused over 12 hours nightly, with a total volume of 1.6 L. He reported 15–30 bowel movements per day. Prior to admission, the patient had a history of central line infections, with six episodes requiring hospitalization and intravenous therapy. In five cases, the causative organisms were identified: *Enterobacter cloacae*, *Staphylococcus hominis* (three episodes), and *Staphylococcus warneri*. He had undergone a total of 13 central line replacement procedures. He was not receiving prophylactic intestinal decontamination therapy. During surgery at our center, an 8 cm ileal reverse segment was created. Postoperatively, he was started on antibiotic therapy with cefazolin and metronidazole three times per day and gentamicin once daily for one week, followed by an oral rifaximin-based decontamination regimen. He was subsequently fitted with a jejunostomy feeding tube (J-PEG) due to retching, refusal of oral nutrition, and delayed gastric emptying.

At his one-year follow-up, the family reported significant improvement, with 5–10 bowel movements per day. His PN regimen was reduced to 1.5 L per night. He weighed 16 kg (still below the 5th percentile) and was 123 cm in height (25th percentile for age).

Case 4

Patient HD is a male born at 36 weeks of gestation with a prenatal diagnosis of gastroschisis, which resulted in vanishing gastroschisis at birth, resulting in the loss of a significant portion of his small intestine. At birth, the patient had only 55 cm of small intestine and 25% of his colon. A tube stoma was created to facilitate controlled bowel expansion, and he subsequently underwent a Small Intestine

Lengthening and Tailoring (SILT) procedure at one year of age. Following surgery, the patient was successfully weaned off PN and transitioned to exclusive enteral nutrition. Nevertheless, he experienced vitamin B12 deficiency due to malabsorption and experienced up to 12 bowel movements per day, along with abdominal distension and pain. At age 15, he underwent additional bowel remodeling surgery due to these persistent symptoms. Intraoperatively, stenosis at the ileocolic anastomosis and bowel dilation were identified and corrected. He was not receiving antibiotic therapy in the immediate preoperative period, and postoperative antibiotic treatment was administered according to the standard protocol for one week. At the one-year follow-up, the patient was tolerating enteral feeding and reported a significant improvement, with 3–5 bowel movements per day. Ongoing follow-up care was coordinated between our center and the referring facility.

Case 5

Patient RB is a male diagnosed with type 3 intestinal failure secondary to Hirschsprung Disease. He was born at term via cesarean section due to fetal malpresentation. The pregnancy initially involved a twin gestation, with intrauterine demise of the co-twin.

After birth, the patient passed meconium only after 48 hours with rectal stimulation. Rectal biopsies confirmed a diagnosis of Hirschsprung Disease. At 3 months of age, he underwent surgery for resection of 50 cm of aganglionic ileum and stoma creation. Histological examination revealed the presence of ganglion cells up to 130 cm proximal to the ileocecal valve. At 4 months, the patient required stoma cerclage due to prolapse, and at 1 year of age, he underwent stoma closure.

At 2 years of age, the patient was referred to our center for bowel assessment and remodeling. He initially underwent STEP and SILT, with 75 cm of remaining small intestine identified during the procedure. Two years later, a pull-through procedure following the Duhamel technique was performed, involving 25% of his remaining colon and the creation of a protective stoma, which was subsequently closed four years later.

At the time of stoma closure, the patient was on an intestinal decontamination regimen, and faecal samples were collected prior to surgery. Postoperatively, he was treated with ceftazidime and metronidazole three times daily for one week. He was receiving PN three nights per week, with a total volume of 800 mL per night. At the time, his weight was 20 kg, placing him below the 3rd percentile for age, while his height was at the 25th percentile.

Case 6

Patient DC is a premature female born at 34 weeks of gestation, with a second-trimester prenatal diagnosis of suspected intestinal volvulus and apple-peel intestinal atresia (IA). At birth, the diagnosis was confirmed as type IIIB IA combined with volvulus, resulting in SBS. She underwent initial surgery

involving jejunal tapering, resection of the atretic segment, and an anastomosis between the tapered jejunum and the terminal ileum.

The patient was initially weaned off PN by the age of 4. During this period, she required multiple central venous catheter (CVC) placements. However, following PN discontinuation, she experienced recurrent episodes of bacterial overgrowth, leading to metabolic acidosis and impaired growth. As a result, PN was reintroduced but later permanently discontinued after the placement of a gastrostomy at the age of 7.

She was referred to our center at the age of 13 due to persistent episodes of metabolic acidosis and imaging findings consistent with significant jejunal dilation and impaired gastric emptying. At the time of referral, she measured 145 cm in height (4th percentile for age) and weighed 30 kg (below the 3rd percentile). She reported 2–3 bowel movements per day and was dependent on enteral nutrition, administered both orally and via gastrostomy, without requiring PN. Her treatment included oral supplementation with vitamins K, A and E.

During bowel assessment, it was determined that the patient retained only 8 cm of small intestine (5 cm of jejunum and 3 cm of ileum) with preservation of the ileocecal valve and 100% of her colon. The left colon was notably dilated, with a diameter of 8 cm. A STEP procedure was performed in this segment using five 55 mm linear stapler firings.

At the time of surgery, the patient was not receiving prophylactic intestinal decontamination. Postoperatively, she was started on an antibiotic regimen and discharged with instructions to follow a rotational decontamination therapy protocol: one week each of metronidazole, ciprofloxacin, and tobramycin, administered alternating cycles.

Case 7

Patient BT is a male born at term via vaginal delivery, with a diagnosis of cloacal exstrophy resulting in short bowel syndrome (SBS). At birth, he retained 70 cm of small intestine and 75% of his colon. He was assigned an XY karyotype and underwent a colostomy shortly after delivery. With parental consent and court authorization, he was assigned female sex, underwent gonadectomy, and a vaginal canal was constructed. Estrogen therapy was initiated as part of the gender assignment process.

Due to significant growth impairment, he was started on PN alongside enteral nutrition. At the age 12, he was referred to our center, where a STEP procedure was performed on a dilated intestinal segment. During his clinical course, he experienced multiple central line complications, including superior vena cava thrombosis and superior caval syndrome with critical stenosis of the superior vena cava following desobstruction. He underwent several interventions, including stent placement and balloon dilation, to manage these complications.

At the age of 18, the patient expressed gender dysphoria and sought to transition back to male sex. Testosterone therapy was initiated at age 19. He also experienced persistent cholestasis secondary to PN, high stomal output (7–8 liters per day), and recurrent hospitalizations for dehydration, electrolyte imbalances, and episodes of D-lactic acidosis.

At age 22, he underwent a second bowel remodeling procedure. At the time of surgery, his treatment included loperamide, ursodeoxycholic acid (Deursil), and monthly intestinal decontamination therapy with rifaximin for one week. Intraoperative findings revealed a markedly dilated distal ileum (up to 10 cm) and cecum (up to 17 cm). Tapering procedures were performed, reducing the ileal diameter to 4 cm and the colonic diameter to 8 cm. Postoperatively, the stomal output significantly decreased; however, the patient was readmitted five months later for surgical correction of a stomal prolapse and again ten months after the initial procedure for cerclage placement using Permacol.

At three-year follow-up, the patient remained on PN for 12 hours per night, supplemented with vitamins and SMOFlipid, with no substantial changes to his preoperative nutritional regimen. In the meantime, he underwent bladder augmentation surgery without complications.

Table 1

Summary table of patients’ clinical details. The table summarizes the clinical characteristics associated with each specific SBS condition for each patient. IF: Intestinal failure; HD: Hirschsprung disease; IA: Intestinal atresia; CE: Cloacal exstrophy; VG: Vanishing gastroschisis. AGIR: autologous gastrointestinal reconstructive surgery. The age at surgery is reported in years.

IF class	Patient	Gender	Age at surgery	Aetiology	Remaining bowel
Type 1	CZ	M	17	HD	Total duodenum and jejunum
Type 2	NF	F	6	Volvulus	5 cm small bowel 75% large bowel
	RS	M	4	IA-IIIa	50 cm small bowel 50% large bowel
	HD	M	15	VG	55 cm small bowel 25% large bowel
Type 3	BT	M	22	CE	70 cm small bowel 75% large bowel
	RB	M	8	HD	75 cm small bowel 25% large bowel
	DC	F	12	IA-IIIb	8 cm small bowel 100% large bowel

DISCUSSION

Changes in blood and urine analytes

Since blood and urine sampling had to be conducted in accordance with the health and well-being status of the subjects, some pre- or post-intervention samples (blood, urine) could not be collected. As a result, not all comparisons are available. When it was possible to carry out the tests, we observed that surgery (*i.e.* the last surgical intervention) affected the level of blood and urine analytes, causing either positive or negative percentage changes when comparing pre- and post-intervention values (Figure S2). Some analytes showed consistent patterns of variation across at least two IF categories, regardless of IF classification. For example, albumin, glucose, potassium, magnesium, and phosphorus in blood displayed similar percentage changes across multiple IF types (Figure S2a). Other changes were specific to individual IF categories: blood chloride levels decreased only in type 2 patients (-5.7%), while sodium levels decreased slightly in type 3 patients (+ 0.6%). Total blood protein levels also showed variable percentage changes, with type 2 patients exhibiting a marked increase (+ 12.7%) compared to types 1 and 3. Urine analytes showed more homogeneous changes after the intervention among the IF classes. The only notable exception was urinary potassium, which increased by 50.7% in type 2, in contrast to the negative variations observed in types 1 and 3 (Figure S2b).

Characterization of gut microbiota profile

Together with the patients' clinical histories, we characterized the intestinal bacterial communities in the faecal samples (See Supplementary methods). Due to the inherent complexity and uneven distribution of samples collected, we were unable to perform a robust stratification based on the time before and after surgical interventions. Nevertheless, given their clinical relevance, we examined temporal fluctuations in the relative abundance of Proteobacteria and Firmicutes. Although both phyla exhibited noticeable oscillations across samples, these variations did not follow a consistent or interpretable pattern in relation to surgical timing (Figure S3). This aspect warrants further investigation and highlights the importance of coordinated, multicentre paediatric studies capable of generating sufficiently powered and temporally balanced datasets to fully elucidate microbiota dynamics surrounding surgical interventions.

We assessed gut microbiota diversity and composition in relation to key SBS patient-specific variables, acknowledging the heterogeneity of individual clinical histories. Samples were then stratified according to residual colonic anatomy and IF classification. Owing to the unequal distribution of samples collected before and after surgery, due to unforeseeable clinical circumstances, analyses were performed exclusively by grouping samples according to the three main categories described above (patient, residual colon anatomy and IF class), with the aim of determining their influence on gut microbiota composition.

The effect of the three main variables on bacterial diversity (based on Aitchison distances) was assessed by using multivariate analysis adonis PERMANOVA fitted using a multifactorial model.

Multivariate analysis revealed that the bacterial diversity was significantly affected by IF category ($R^2 = 0.164$, $p < 0.001$), residual colon anatomy ($R^2 = 0.118$, $p < 0.001$), and patient specific history ($R^2 = 0.290$, $p < 0.001$) (Table S1). Together, all the three variables accounted for 57.1% of the variation observed in the bacterial community structure, meaning that more than half of the differences detected among samples can be attributed directly to the variables included in the model. As expected, the greatest effect was associated with the *patient* variable, *i.e.* specific clinical history, who, besides further stratification methods, showed that patient medical history dramatically impacted the observed diversity. We tested the dispersion effect, showing that dispersion differed significantly across patients ($p = 0.007$), indicating heterogeneity in community variability among subjects, expected and related to the nature of the sampling dataset. The PCA on CLR-transformed ASVs showed clear samples separation according to the *patient* and, to a lesser extent, to the *IF class* variables (Figs. 1a,c), in accordance with the effect highlighted by adonis PERMANOVA. We also tested the effect of residual colon anatomy as a continuous variable through environmental fitting analysis (Fig. 1d). The environmental fitting analysis showed a strong linear association with ordination (*envfit*. $R^2 = 0.506$, $p < 0.001$), and the ordisurf model revealed a smooth gradient of percentage across the PCA space, particularly aligned with the PC1 axis, highlighting that bacterial diversity changed following the gradient the percentage of residual colon anatomy (Fig. 1d). Pairwise Sørensen distances, combined with mean inter-group distances, revealed marked heterogeneity in bacterial community composition among patients. Sørensen distances quantify the fraction of non-shared taxa between two communities, whereas the mean distances summarize the overall dissimilarity of a given patient group relative to all other groups. The observed pairwise distances ranged from 0.32 to 0.97, indicating that certain patients (*e.g.*, RS and RB) presented highly distinct bacterial assemblages, while others (*e.g.*, BT and NF) display comparatively moderate dissimilarity (Fig. 1b).

Analysis of mean distances further refines this pattern. Group BT exhibited a moderate mean distance to all other groups (mean = 0.88), with relatively low variance across pairwise comparisons, indicating that its bacterial composition is moderately divergent but internally consistent relative to the broader patient cohort. In contrast, groups such as RS demonstrated both high mean distances (mean = 0.94) and a broader distribution of pairwise distances, signifying that these patients harbor bacterial communities that are compositionally distinct and more variable relative to other groups (Fig. 1b).

These patterns allow preliminary classification of patient groups based on bacterial similarity: (i) a cluster of more similar communities, including BT, NF, and RB, which share moderate dissimilarity values and relatively low intra-group variability; (ii) a set of highly distinct communities, such as RS and DC, characterized by elevated mean distances and broader variability; and (iii) intermediate groups (*e.g.*, CZ and HD) showing moderate mean distances and intermediate heterogeneity.

Alpha diversity analysis showed that the total number of bacterial ASVs (also referred to as observed richness) showed significant differences across patients ($p = 0.0017$), while Shannon, Simpson, and Evenness indices did not display significant variation (all $p > 0.18$) (Fig. 1e). Tukey post-hoc tests for

observed richness identified multiple pairwise patient differences, highlighting a significantly higher number of bacterial ASVs in patient CZ compared to all other subjects, except for patient BT (Fig. 1e).

The Likelihood Ratio Test (LRT of DESeq2) identified 15 ASVs that significantly changed among patients ($\text{padj} < 0.05$). Clustering analysis performed on the differential abundances of ASVs significantly selected by LRT revealed a clustering consistent with *patient* variable, highlighting distinct abundance patterns (Fig. 2). The presence of a polarized distribution, i.e. highly abundant ASVs in some subjects but almost absent in others, reflected the marked interindividual variability of the subjects' microbiota highlighted in the diversity analyses.

The distribution of ASVs exhibited a marked polarization, i.e. with some variants highly abundant in certain subjects and nearly absent in others. In particular, three *Lactobacillus* variants, likely corresponding to distinct species, displayed clearly differentiated distribution patterns across individuals (Fig. 2). ASV 2 was highly represented in subjects NF, DC, and BT, showed lower scaled abundance in HD and CZ, and was almost completely absent in RB and RS. Conversely, RB was characterized by a pronounced predominance of *Lactobacillus* ASV 1 (Fig. 2). Moreover, *Lactobacillus* ASV 15 was detected exclusively in the BT patient and was absent in all other subjects (Fig. 2). Additional notable patterns included ASV 3 (*Escherichia-Shigella*), which was relatively evenly distributed across the dataset except for subject RS, who showed a marked enrichment of ASV 10 (*Morganella*). ASV 5 (*Enterococcus*) was more abundant in CZ, RB, and NF, whereas ASV 28 (*Bacteroides*) was identified exclusively in BT. Collectively, these patterns, described as polarizations due to their near-binary distribution, highlight the substantial inter-individual variability attributable to the subjects' clinical histories, IF conditions, and residual intestinal anatomy, all of which drastically impact in selecting specific bacterial variances.

CONCLUSION

This study presents a comprehensive case series of seven paediatric patients diagnosed with SBS, highlighting the broad heterogeneity in etiologies, surgical histories, and clinical outcomes associated with this complex condition. The age distribution within the cohort reflects the chronic and progressive nature of SBS, with most patients requiring prolonged medical and surgical interventions from infancy through adolescence.

Our results indicate that surgical interventions can lead to significant changes in blood and urine analytes, suggesting that surgical remodeling may influence metabolic parameters and overall nutritional status. For instance, the observed variations in blood levels of albumin, glucose, potassium, magnesium, and phosphorus across different IF categories underscore the importance of monitoring these parameters in managing SBS according to the IF classification. In particular, the variations in electrolytes and proteins, especially the increase in blood protein levels in type 2 patients, may reflect a compensatory mechanism in response to surgical stress or changes in nutritional support. Moreover, the specific alterations in blood chloride and sodium levels observed in IF type 2 and type 3 patients,

respectively, likely represent distinct metabolic disturbances associated with each classification, warranting further investigation into their clinical significance.

By correlating IF types with distinct fecal microbiota profiles, our findings underscore the potential of microbiota analysis as a valuable tool for characterizing the clinical and nutritional status of SBS patients. The observed microbial differences among IF types suggest that gut microbiota composition reflects the degree of intestinal adaptation and functionality, offering a microbiological lens through which to interpret disease progression.

Looking ahead, integrating microbiota profiling with metabolic characterization may further enhance our ability to define the malnutrition status of patients with different forms of intestinal failure. This combined approach could support more accurate risk stratification, guide personalized nutritional and therapeutic interventions, and ultimately improve patient outcomes. The IF classification, when interpreted alongside microbial and metabolic data, could evolve into a more comprehensive framework for the clinical management of pediatric SBS, paving the way for precision medicine strategies in this vulnerable population.

Abbreviations

ASVs

Amplicon Sequence Variants

CVC

central venous catheter

GI

gastrointestinal

HD

Hirschsprung Disease

IA

Intestinal Atresia

IF

Intestinal Failure

IUGR

intrauterine growth restriction

J-PEG

jejunostomy feeding tube

NRS

Numerical Rating Scale

PN

Parenteral Nutrition

SBS

Short Bowel Syndrome

SILT
Small Intestine Lengthening and Tailoring
STEP
Serial Transverse Enteroplasty
VG
Vanishing Gastroschisis

Declarations

Availability of data and material

Raw data from 16S (V3-V4) rRNA gene sequencing were uploaded to the European Nucleotides Archive (ENA) under project ID PRJEB71626. Additional information can be provided upon reasonable request to the corresponding authors.

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Authors' contributions

RC, CDF and AM: conceptualization of the work. CO and EL: sample collections and investigation. RC, NM, FG, SC, MMM, CO and CDF: writing-original draft and interpretation of the data. AM, CDF and DC:

critical revision of the article for important intellectual content. SR and BC: sample sequencing. NM: formal analysis. AM and CDF: funding acquisition.

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Ethical approval and consent to participate

The Ethics Committee of the Meyer Children's Hospital IRCCS granted ethical clearance for this study (protocol number GDF15).

Consent for publication

The data reported were obtained with informed consent and were reported in anonymous format.

Competing interests

The authors declare that they have no conflict of interest.

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Table 2

Table 2 is not available with this version.

Figures

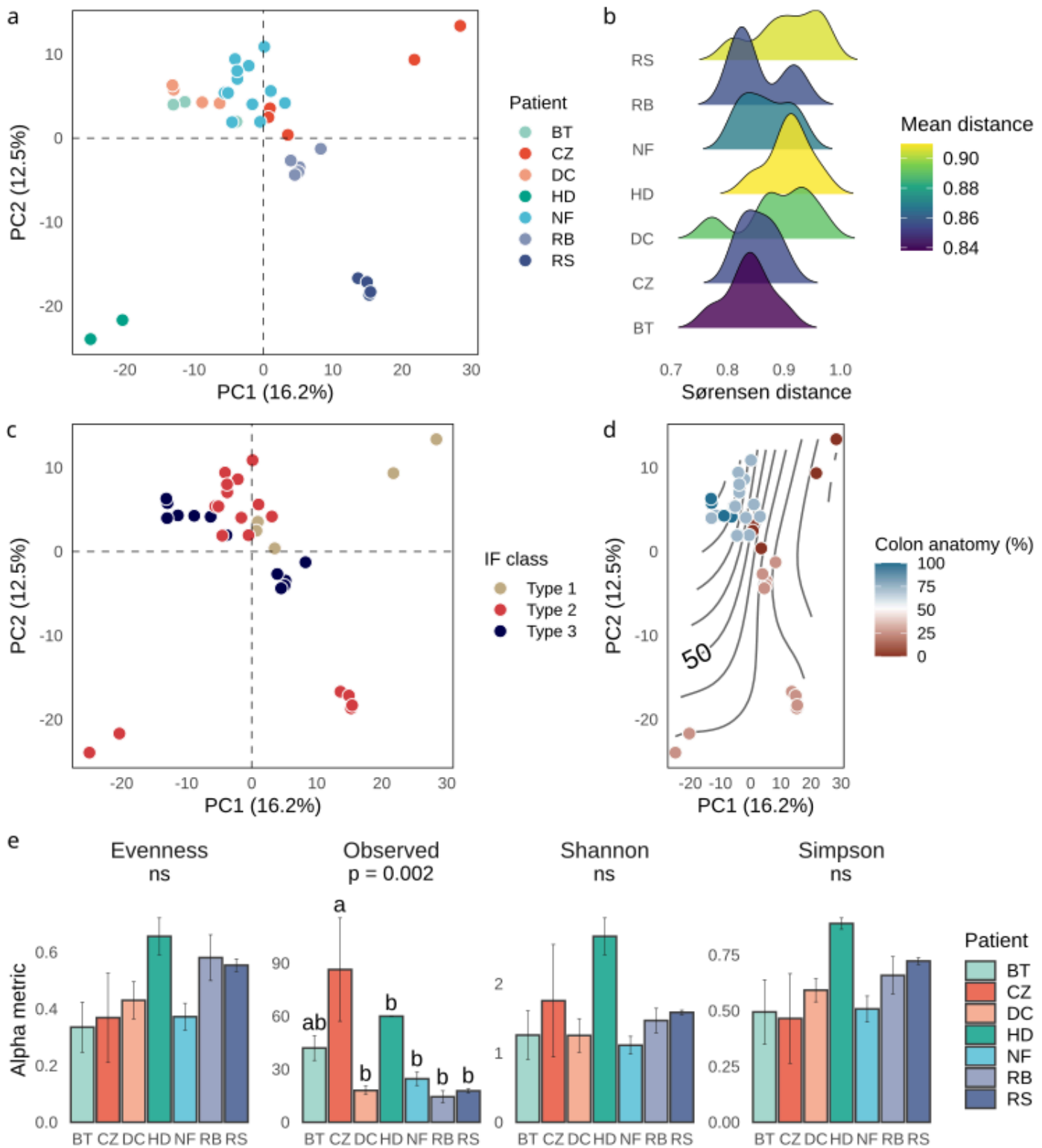


Figure 1

Differences in bacterial diversity among patients. (a,c) PCA based on Aitchison distances reports the sample distribution according to the patient (a) and IF classification (c) variables. Samples are colored by patient or IF class according to the color scheme reported in the legends. (b) The ridgeline density plot represents the distribution of pairwise Sørensen distances for a given source group (y-axis) against all other target groups (x-axis). The fill color corresponds to the mean inter-group distance of the source

group, with darker shades indicating higher overall dissimilarity. This visualization allows assessment of both the variability of distances within each group and the relative distinctness of microbial community compositions among groups. (d) Ordurf surface fitting illustrates the continuous effect of residual colon anatomy (expressed as %) across the PCA space. Residual colon anatomy gradient is highlighted by using color gradient in the legend. (e) Barplot reports differences in alpha diversity metrics (Observed, Shannon, Simpson and Evenness) among patients. Significant differences are assessed by using Anova test fitted using linear model and post-hoc pairwise comparisons among patients were performed using Tukey's HSD test. Lowercase letters on top of each bar represent significant differences across patients: if significantly different, all letters on top of the two boxes must be different; if not significantly different, at least one letter must be the same.

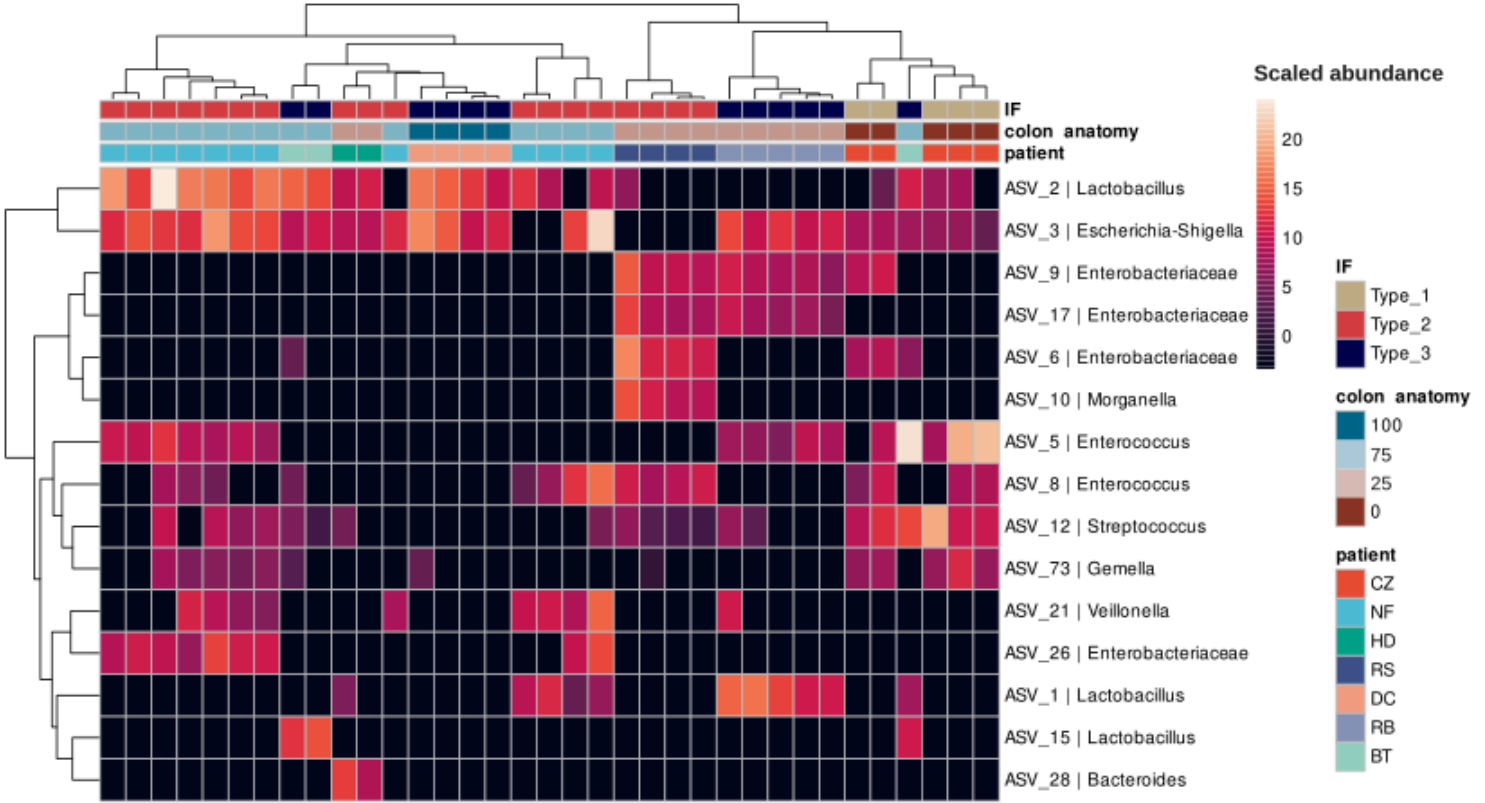


Figure 2

ASVs differentially represented among patients.The heatmap reports the VST-normalized values of the ASVs significantly selected by LRT of DESeq2 ($\text{padj} < 0.05$) across patients. Rows represent ASVs labeled with the deepest available taxonomic assignment while columns represent samples. Samples are annotated by patient, residual colon anatomy, and IF classification and reported using color scheme in the legend. Row and column clusters were generated using euclidean distance and the Ward D2 method. The color scale indicates normalized abundance: high values are blue, low values are light red.

Supplementary Files

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- [ColettaetalSupplementarymaterialrevised2.docx](#)