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# Gut Colonization Preceding Mucosal Barrier Injury Bloodstream Infection in Pediatric Hematopoietic Stem Cell Transplantation Recipients

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A B S T R A C T

The gastrointestinal tract is the predicted reservoir for most bloodstream infections (BSIs) after hematopoietic stem cell transplantation (HSCT). Whole-genome sequencing and comparative genomics have the potential to improve our understanding of the dynamics of gut colonization that precede BSI in HSCT recipients. Within a prospective cohort study of children (age <18 years) undergoing HSCT, 9 subjects met criteria for mucosal barrier injury BSI. We performed whole-genome sequencing of the blood culture isolate and weekly fecal samples preceding the BSI to compare the genetic similarity of BSI isolates to fecal strains. We evaluated temporal associations between antibiotic exposures and the abundances of BSI strains in the gut microbiota and correlated the detection of antibiotic resistance genes with the phenotypic antibiotic resistance of these strains. The median patient age was 2.6 years, and 78% were male. BSIs were caused by *Escherichia coli* (n = 5), *Enterococcus faecium* (n = 2), *Enterobacter cloacae* (n = 1), and *Rothia mucilaginosa* (n = 1). In the 6 BSI episodes with evaluable comparative genomics, the fecal strains were identical to the blood culture isolate (>99.99% genetic similarity). Gut domination by these strains preceded only 4 of 7 *E. coli* or *E. faecium* BSIs by a median of 17 days (range, 6 to 21 days). Increasing abundances of the resulting BSI strains in the gut microbiota were frequently associated with specific antibiotic exposures. *E. cloacae* and *R. mucilaginosa* were not highly abundant in fecal samples preceding BSIs caused by these species. The detection of antibiotic resistance genes for $\beta$-lactam antibiotics and vancomycin predicted phenotypic resistance in BSI strains. Bacterial strains causing mucosal barrier injury BSI in pediatric HSCT recipients were observed in the gut microbiota before BSI onset, and changes in the abundances of these strains within the gut preceded most BSI episodes. However, frequent sampling of the gut microbiota and sampling of other ecological niches is likely necessary to effectively predict BSI in HSCT recipients.



## INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is used to treat a growing number of malignant and nonmalignant conditions in children and adults. The conditioning chemotherapy that patients receive in preparation for HSCT results in prolonged neutropenia and injury to mucosal surfaces, facilitating translocation of microbes across these barriers and predisposing to bloodstream infection (BSI). The most frequent causes of BSI in HSCT recipients are Enterobacteriaceae, enterococci, and *viridans* group streptococci [1,2]. These infections are associated with substantial morbidity and mortality, with case fatality rates of 8% to 67%, depending on the causative organism [2,3]. The gastrointestinal tract is the purported source of most of these infections, which suggests that serial monitoring of the gut microbiota could identify patients who are at high risk for BSI after HSCT. However, previous studies were limited by infrequent fecal sampling or the use of low-resolution methods (eg, pulsed-field gel electrophoresis) to compare the genetic similarity of blood and fecal strains [4-7].

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Whole-genome sequencing recently emerged as a tool that provides unparalleled resolution for comparative microbial genomics and enables confident determination of clonal relationships [8].

In this study, we performed whole-genome sequencing of fecal samples and blood culture isolates for BSI episodes occurring in a cohort of pediatric HSCT recipients. We compared the genetic similarity of fecal strains with the BSI strains and determined whether shifts in the relative abundances of these strains in the gut microbiota preceded BSI episodes. As secondary objectives, we evaluated associations between antibiotic exposures and the abundances of BSI strains in the gut microbiota and correlated the detection of antibiotic resistance genes with the phenotypic antibiotic resistance of these strains.

## METHODS

### *Study Population*

Eligible subjects were age <18 years and undergoing evaluation for their first allogeneic or autologous HSCT at Duke University between October 2015 and February 2018. One hundred subjects were enrolled and followed from before HSCT up to 100 days after HSCT. For *Pneumocystis jirovecii* prophylaxis, subjects received trimethoprim-sulfamethoxazole starting at hospital admission and continuing until 2 days before HSCT, followed by inhaled or i.v. pentamidine starting 30 days after HSCT. Routine antibacterial prophylaxis was not administered. The analyses presented herein were limited to subjects who developed a mucosal barrier injury-related laboratory-confirmed BSI, as defined by the US National Healthcare Safety Network [9].

### *Data and Specimen Collection*

Sociodemographic and clinical data were collected from a caregiver questionnaire and medical record review. Fecal samples were collected into vials containing RNAlater solution (Thermo Fisher Scientific, Waltham, MA) and immediately refrigerated at 4°C. Study staff transported these samples daily (Monday through Friday) from clinical sites to the laboratory for storage at -80°C. Blood culture isolates were obtained from the Duke University Clinical Microbiology Laboratory. The laboratory routinely stores bacterial isolates from blood cultures for which there is monomicrobial growth in glycerol at -80°C. Bacterial isolates are tested for susceptibility to antimicrobials using the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Berkeley, CA), and breakpoints are provided by the Clinical and Laboratory Standards Institute [10]. For BSI episodes occurring in enrolled subjects, blood culture isolates were plated on blood agar, and single colonies were selected for whole-genome sequencing.

The dataset supporting the conclusions of this study is available in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/bioproject/524322). The statistical code used for data analyses is also publicly available (https://gitlab.com/buccilab_public/pediatric_bsi).

### *DNA Extraction and Whole-Genome Sequencing*

We selected weekly fecal samples from study enrollment to the BSI episode for DNA extraction and whole-genome sequencing. Total genomic DNA was extracted from fecal samples and blood culture isolates using a modified version of the Earth Microbiome Project protocol, as described previously [11,12]. DNA sequencing libraries were constructed using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA) and sequenced on a NextSeq500 Sequencing System as 150-base paired-end reads. Sequences were trimmed and removed of host contamination using Trimmomatic [13] and Bowtie [14]. Host-decontaminated reads were then profiled for bacterial species abundances using MetaPhlAn2 [15,16]. Abundances of antibiotic resistance genes were determined by mapping trimmed and host-decontaminated reads to the Comprehensive Antibiotic Resistance Database (CARD) using the ShortBRED bioinformatics pipeline [17,18]. Normalized taxonomic and resistance gene abundances were then used for downstream analyses and visualization in R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

### *Strain Variant Identification*

Genomic assemblies were constructed for blood culture isolates using the SPAdes-3.11 assembler [19]. Multilocus sequence typing assignments were determined from the assembled contigs using mlst (https://github.com/tseemann/mlst). Based on the assignments, representative references were chosen for each species (*Escherichia coli*, accession HG941718.1; *Enterococcus faecium*, accession NC_017960.1). Paired-read data from both the blood culture isolate and fecal samples were aligned to these references using Bowtie2 [14] and SAMtools [20], and single nucleotide variants were detected with Pilon [21]. Samples in which aligned reads obtained passing coverage of at least 80% of the total reference genome were included in subsequent strain-level analyses. This resulted in comparison of *E. coli* strains over >3.8 Mbp and *E. faecium* strains over >2.4 Mbp, comprising 75% and 90% of the genomes for these species, respectively. Sequencing reads from fecal samples were used to draw a consensus conclusion about the genomic sequence of the dominant strain in the gut microbiota. Custom scripts were applied to determine conserved core nucleotide positions and produce multi-FASTA alignments. Core single nucleotide variant positions were determined from a multisequence alignment file of all core genome sequences using *SNP-sites* [22]. RAxML version 8.2.10 [23] and FigTree version 1.4.3 [24] were used to generate and format the phylogenetic trees.

## RESULTS

### *Study Population*

During the study period, 14 mucosal barrier injury BSIs occurred in the study population. For 3 BSIs, the blood culture isolate was not frozen by the microbiology laboratory or did not grow from the stored sample. No fecal samples were available preceding 2 additional BSI episodes. The final dataset comprised 9 mucosal barrier injury BSI episodes. Characteristics of the subjects and BSI episodes are presented in Table 1. Most subjects were male and had a malignant HSCT indication, and nearly one-half were recipients of an umbilical cord blood transplant. The most frequent BSI organisms identified were *E. coli* (n = 5) and *E. faecium* (n = 2).

### *Whole-Genome Sequencing*

A total of 229,427,210 high-quality bacterial sequencing reads (mean of 4,498,573 sequences per sample) were obtained from the 42 fecal samples and 9 blood culture isolates included in these analyses. The median (interquartile range) number of reads per fecal sample was 2,715,388 (815,979–5,006,084). The median (interquartile range) number of reads per BSI isolate was 11,195,760 (6,667,320–14,876,920), with a median of 177-fold genome coverage depth. Sequences were assigned to 199 bacterial species, representing 74 genera from 6 phyla. The relative abundances of frequently occurring species in fecal samples are shown in Supplemental Figure 1.

**Table 1**
Characteristics of the Study Population (n = 9)

| Characteristic | Value | |
|---|---|---|
| Age, yr, median (IQR) | 2.6 | (2.0-6.9) |
| Sex, n (%) | | |
| Female | 2 | (22) |
| Male | 7 | (78) |
| Race/ethnicity, n (%) | | |
| Black or African-American | 1 | (11) |
| Non-Hispanic white | 4 | (44) |
| Middle Eastern or Arab-American | 3 | (33) |
| Native American or Alaskan Native | 1 | (11) |
| Indication for HSCT, n (%) | | |
| Hematologic malignancy | 2 | (22) |
| Genetic or metabolic disorder | 2 | (22) |
| Nonmalignant hematologic disorder | 2 | (22) |
| Solid tumor | 3 | (33) |
| HSCT type and source, n (%) | | |
| Allogeneic | | |
| Matched, related bone marrow | 1 | (11) |
| Umbilical cord blood | 4 | (44) |
| Autologous | 4 | (44) |
| BSI organism, n (%) | | |
| *Enterobacter cloacae* | 1 | (11) |
| *Enterococcus faecium* | 2 | (22) |
| *Escherichia coli* | 5 | (56) |
| *Rothia mucilaginosa* | 1 | (11) |

IQR, interquartile range.

### *Fecal Strains Are Identical to Subsequent BSI Isolates*

The genetic relatedness of fecal strains to blood culture isolates is shown in Table 2. For all BSI episodes for which comparative genomics was possible, the dominant strain within one or more fecal samples was identical (>99.99% genetic similarity) to the blood culture isolate. Moreover, within an individual patient, strains from fecal samples collected closer to the BSI episode had fewer nucleotide differences relative to the BSI isolate (Patients 2 and 7). Phylogenetic trees derived from core genomic sequences of *E. coli* and *E. faecium* are shown in Figure 1. Blood culture isolates and fecal strains clustered by patient. The lone exception was an *E. coli* strain identified in a fecal sample obtained 34 days before the BSI episode (-34d) from patient 7, which suggests that the BSI strain replaced the previous *E. coli* strain in the gut of this patient between -34d and -25d.

### *Relative Abundances of Strains in the Gut Microbiota Increase Before BSI Onset*

Supplementary Figure 2 shows the Shannon Diversity Index of fecal samples relative to the timing of BSI. In most patients, the BSI episode was preceded by a loss of gut microbial diversity, which often corresponded to a high or rising relative abundance of the BSI species (Figure 2). We observed several patterns of pathogen presence in the gut microbiota preceding BSI. First, gut domination by the BSI species, defined as a relative abundance of at least 30% in the gut microbiota, preceded 4 of 7 BSI episodes caused by *E. coli* or *E. faecium* for a median of 17 days (range, 6 to 21 days) [6]. Second, gut domination did not occur in patients 4 and 6, but both BSI episodes were closely preceded by rapid increases in the relative abundance of the BSI species in the gut microbiota. The abundance of *E. coli* rose from 0% (-4d) to 29% (-1d) in patient 4 and from 0.2% (-10d) to 11% (-2d) in patient 6. Third, patient 8 developed an *E. coli* BSI following a stable, moderate abundance (4% to 12%) of this species in the gut microbiota between -18d and -1d. Finally, the BSIs caused by *E. cloacae* and *R. mucilaginosa* were preceded by low abundances of these species in the gut microbiota. In patient 1, the *E. cloacae* relative abundance was <2% in serial fecal samples obtained between -20d and -1d. *R. mucilaginosa* was not detected in fecal samples obtained from patient 9 on -15d and -4d.

**Table 2**
Genomic Concordance of Fecal Strains to Blood Culture Isolates

| Patient | BSI Organism | Day of Fecal Sampling | SNVs |
|---|---|---|---|
| 2 | *Enterococcus faecium* | -7 | 46 |
| | | -4 | 40 |
| 3 | *E. faecium* | -11 | 12 |
| 5 | *Escherichia coli* | -20 | 0 |
| | | -1 | 0 |
| 6 | *E. coli* | -4 | 5 |
| 7 | *E. coli* | -34 | 826 |
| | | -25 | 1 |
| | | -16 | 0 |
| | | -7 | 0 |
| | | -1 | 0 |
| 8 | *E. coli* | -6 | 2 |

SNVs, single-nucleotide variants.
For *E. faecium* isolates, a total of 2,436,174 core bases were compared with the reference genome. For *E. coli* isolates, 3,848,800 core bases were compared with the reference genome.

### *Detection of Antibiotic Resistance Genes and Phenotypic Resistance of BSI Isolates*

The results of antibiotic susceptibility testing of blood culture isolates are presented in Table 3. Both *E. faecium* isolates were resistant to vancomycin. The *E. cloacae* strain and 3 *E. coli* isolates (patients 5, 7, and 8) were resistant to third-generation cephalosporins. The detection of antibiotic resistance genes in blood culture isolates is shown in Figure 3. The *vanA* gene cluster was detected from both vancomycin-resistant *E. faecium* (VRE) isolates. Genes encoding extended-spectrum $\beta$-lactamases (ESBLs; TEM-211, CTX-M-72, CTX-M-100, CTX-M-110, and OXA-1) were detected from the *E. cloacae* and *E. coli* isolates with resistance to third-generation cephalosporins. Resistance genes for aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole were frequently detected but were not strongly correlated with phenotypic resistance in the *E. cloacae* and *E. coli* isolates.

### *Rising Abundances of BSI Strains Were Frequently Associated with Antibiotic Exposures*

In the majority of BSI episodes, receipt of specific antibiotics was associated with changes in the relative abundance of the BSI strain in the gut microbiota (Figure 2). Specifically, there was a temporal association between a rise in the abundance of the BSI strain in fecal samples and exposure to an antibiotic to which this strain was resistant. In patients 2 and 3, an increase in the relative abundance of the VRE strain in the gut microbiota was observed during exposure to vancomycin. In patient 5, the relative abundance of *E. coli* in fecal samples increased from 12% (-12d) to 94% (-1d) while the patient was receiving cefepime for empirical treatment of febrile neutropenia. In patient 6, the increase in abundance of *E. coli* from .2% (-10d) to 11% (-2d) occurred during treatment with trimethoprim-sulfamethoxazole. Finally, in patient 7, exposure to piperacillin-tazobactam and subsequently moxifloxacin correlated with an increase in the abundance of *E. coli* from 18% (-25d) to 75% (-1d).

### *Gut Colonization Did Not Precede Non-Mucosal Barrier BSI*

To evaluate whether the gut might serve as a reservoir for pathogens that are not classified as mucosal barrier injury organisms, we obtained the blood culture isolate for 3 patients with BSI episodes caused by *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Stenotrophomonas maltophilia*. We performed whole-genome sequencing of the blood culture isolates and the fecal sample most closely preceding the BSI (*P. aeruginosa*, -1d; *S. aureus*, -2d; *S. maltophilia*, -4d). In each of these cases, we identified no sequencing reads in the fecal sample corresponding to the BSI species. The sequencing depth for these fecal samples (mean of 5,021,156 sequences per sample) was comparable to that for the fecal samples included in comparative genomics analyses of mucosal barrier injury BSI.

## DISCUSSION

Using intensive prospective fecal sampling and high-resolution comparative genomics, this study demonstrates conclusively that the gut microbiota is a reservoir for bacterial strains that cause BSI after HSCT. This study also provides detailed information about pathogen dynamics within the gut that will inform future studies seeking to use applied microbiomics to predict and prevent BSI in HSCT recipients.

Previous studies in HSCT recipients focused primarily on a relative abundance threshold in the gut microbiota that predisposes to BSI [6,25,26]. Most notably, Taur et al. [6] established the concept of domination to represent when a single

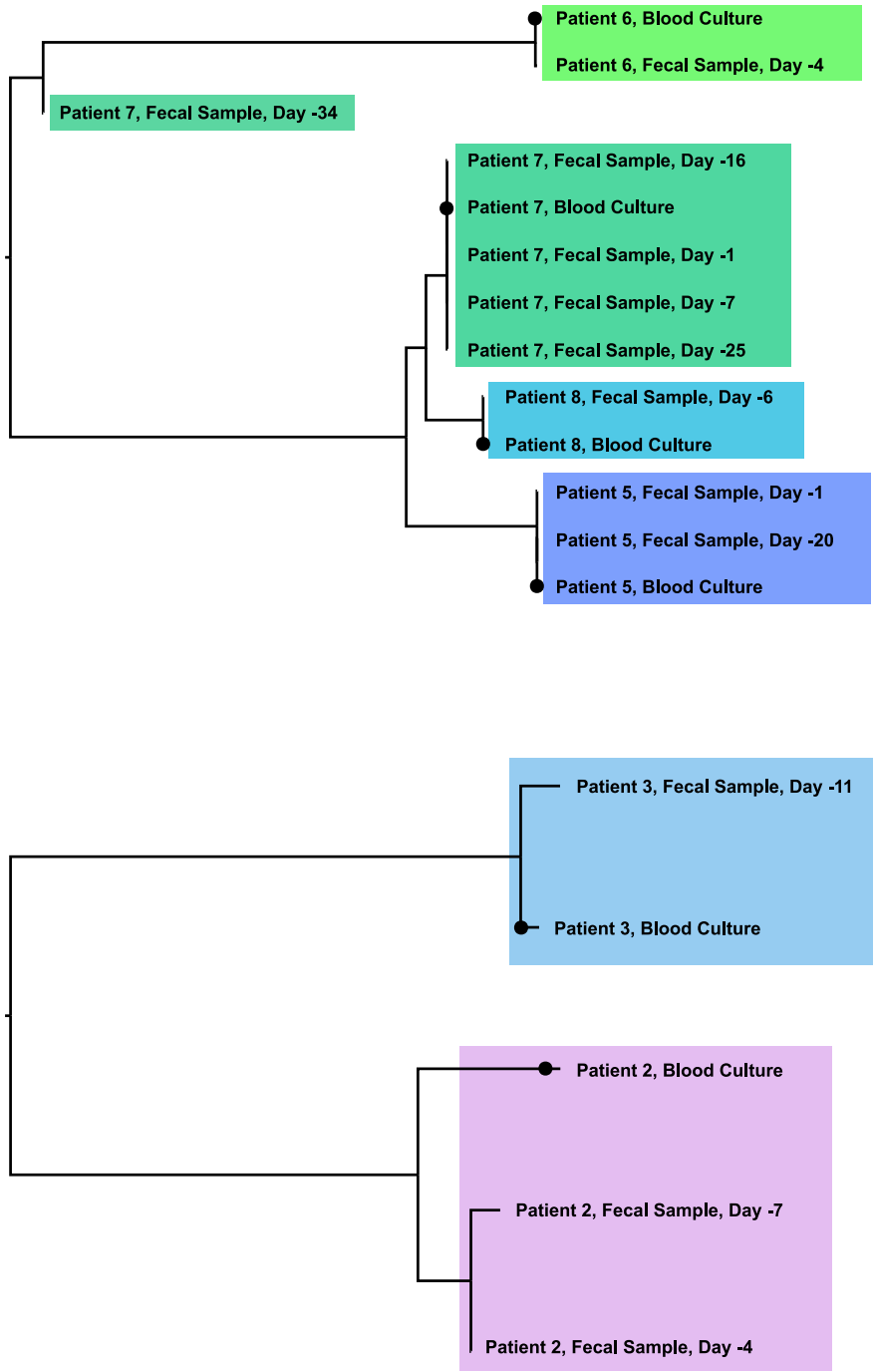


**Figure 1.** Phylogenetic trees for *E. coli* and *E. faecium* strains. Core, single nucleotide variant phylogeny of *E. coli* and *E. faecium* is presented for blood culture isolates and corresponding fecal strains. Strains are color-coded according to patient, and the sample day is presented relative to the BSI episode. Strains from a single patient are more closely phylogenetically related compared with strains from different individuals.

bacterial taxon comprises 30% or more of the gut microbiota and demonstrated that domination by Proteobacteria or *Enterococcus* was associated with BSI caused by these bacteria in adult HSCT recipients. In support of these findings, more than one-half of the BSI episodes caused by *E. coli* and VRE in our cohort were preceded by gut domination by the identical strain for a median of 17 days. This was particularly striking for VRE, which composed >99% of the gut microbiota in fecal samples preceding BSI episodes caused by this species.

In contrast, several patients developed *E. coli* BSI following a rapid rise in the relative abundance of *E. coli* in the gut microbiota in the absence of prolonged gut domination. Most strikingly, 1 patient developed an *E. coli* BSI following an increase in the relative abundance of *E. coli* in the gut microbiota from 0% to 29% over only 3 days. Similarly, another patient developed an *E. coli* BSI after the relative abundance of *E. coli* rose from 0.2% to 11% over an 8-day period. This suggests that a rapid rise in the relative abundance of a strain in the gut microbiota may be an important harbinger of BSI, even if this strain is not highly abundant. Moreover, the rapidity of the shifts in gut microbiota composition after HSCT illustrates that frequent fecal sampling will be a

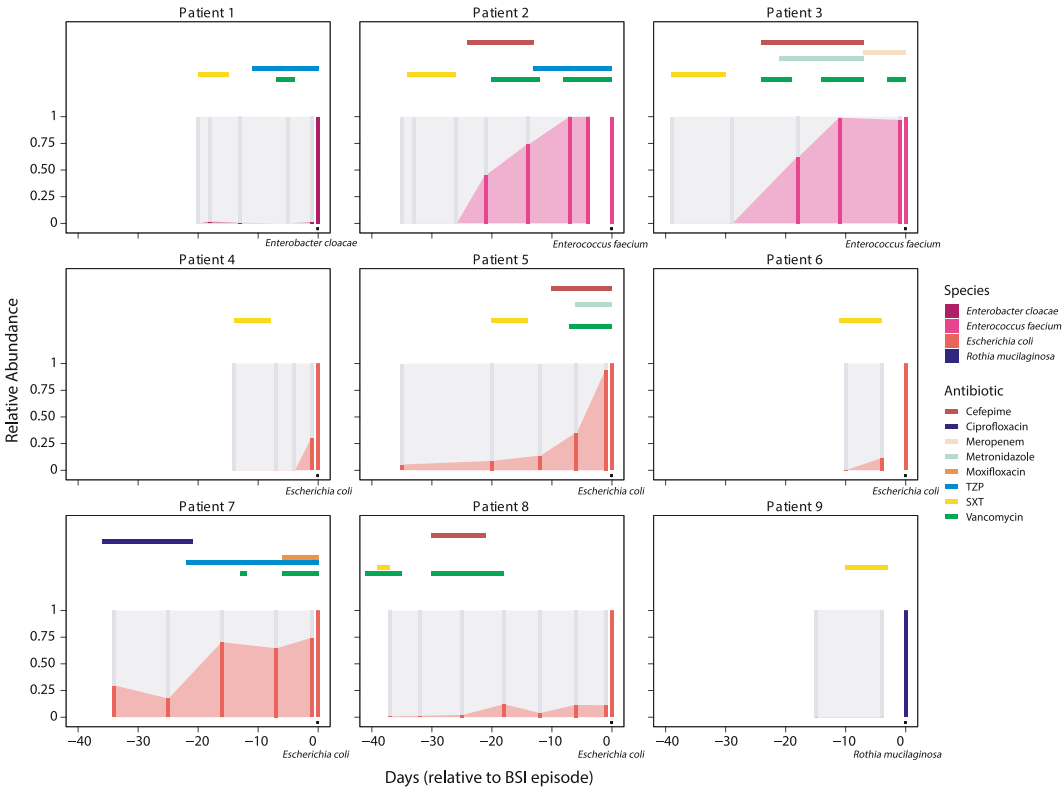


**Figure 2.** Relative abundances of bacterial species in the gut microbiota preceding BSI onset. Bacterial reads were classified at the species level, and the relative abundance of the BSI species in fecal samples is shown preceding the BSI episode. Each darkened vertical bar corresponds to the relative abundance of the BSI species in a single fecal sample. Shading between each bar is provided to visualize changes in relative abundance between fecal samples. The blood culture isolate is shown as a vertical bar and black circle on day 0. Specific antibiotic exposures are depicted as horizontal bars above each plot, with the position and length of the bar corresponding to the timing and duration of the antibiotic exposure. TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole.

necessary component of strategies seeking to use the gut metagenome for the prediction of BSI and other clinical outcomes in HSCT recipients.

Our results also indicate that serial monitoring of the gut microbiota is unlikely to be effective in predicting BSI episodes caused by organisms that do not typically have an ecological niche within the lower gastrointestinal tract. For instance, a patient in our cohort developed a BSI caused by *R. mucilaginosa* despite the absence of this species in several fecal samples closely preceding the BSI episode. *Rothia* species are typically abundant in the oral microbiota but are infrequently detected in samples from the lower gastrointestinal tract [27-29]. Interestingly, this patient had severe oral mucositis coinciding with the onset of the BSI episode. Moreover, we did not detect the BSI species in fecal samples preceding infections caused by several non-mucosal barrier injury pathogens (ie, *P. aeruginosa, S. aureus*, and *S. maltophilia*). Although each of these species was previously shown to colonize the gut of HSCT recipients, the predominant ecological niches for these organisms are the respiratory tract and skin [30,31]. Tamburini et al. [7] recently reported that strains of *P. aeruginosa* and *Staphylococcus epidermidis* detected in fecal samples were highly genetically similar to the blood culture isolates from BSI episodes occurring in adult HSCT recipients. Notably, these strains were of low relative abundance (<3%) in the gut microbiota of these patients, and these species were not detected in fecal samples from several other patients who developed BSI episodes caused by these species. Thus, although it seems

**Table 3**
Antibiotic Susceptibility Testing of Blood Culture Isolates

| Patient | BSI Organism | AMP | CRO | CEF | TZP | MEM | TOB | CIP | SXT | TCN | VAN |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Enterobacter cloacae* | R | R | S | S | S | S | S | R | S | - |
| 2 | *Enterococcus faecium* | R | - | - | - | - | - | - | - | - | R |
| 3 | *E. faecium* | R | - | - | - | - | - | - | - | - | R |
| 4 | *Escherichia coli* | S | S | S | S | S | S | S | S | S | - |
| 5 | *E. coli* | R | R | R | S | S | R | R | R | S | - |
| 6 | *E. coli* | R | S | S | S | S | S | S | R | S | - |
| 7 | *E. coli* | R | R | R | R | S | R | R | S | S | - |
| 8 | *E. coli* | R | R | R | S | S | I | R | R | S | - |
| 9 | *Rothia mucilaginosa* | S | - | - | - | - | - | R | R | - | S |

AMP, ampicillin; CRO, ceftriaxone; CEF, cefepime; TZP, piperacillin-tazobactam; MEM, meropenem; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; TCN, tetracycline; VAN, vancomycin; S, susceptible; I, intermediate; R, resistant.
Susceptibility of bacterial species to specific antibiotics was determined using breakpoints provided by the Clinical and Laboratory Standards Institute.

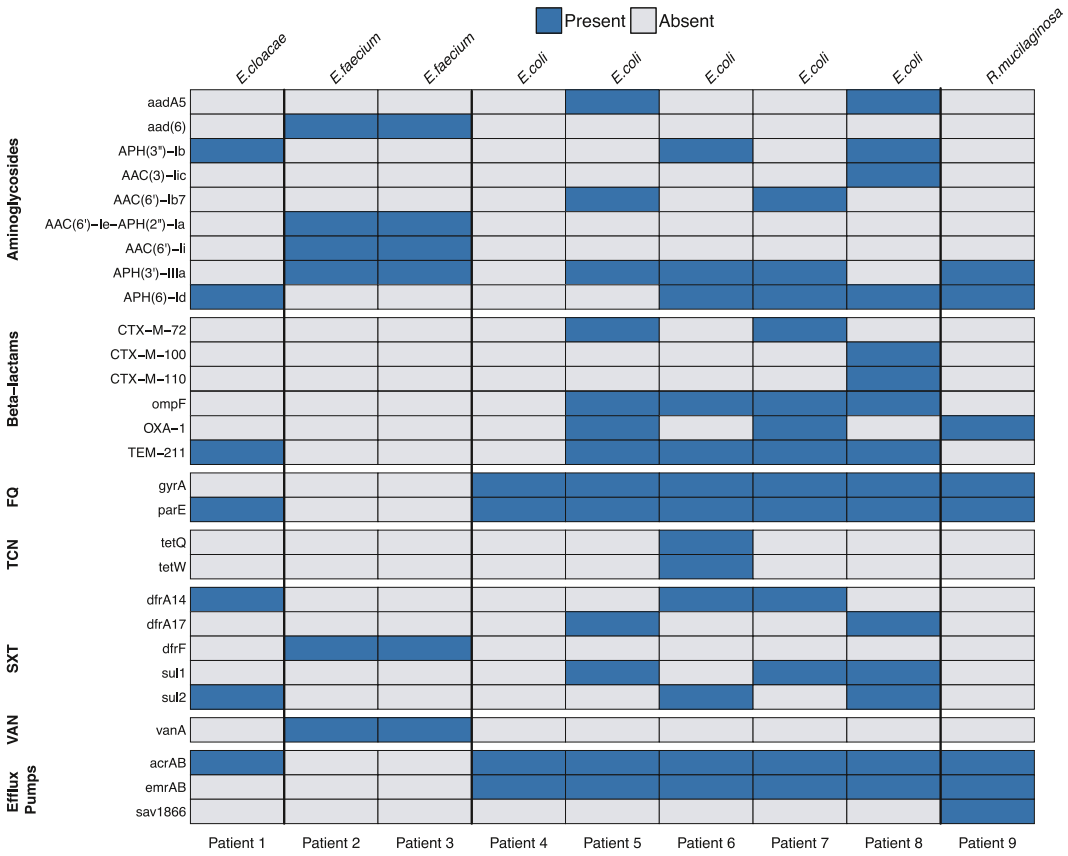


**Figure 3.** Detection of antibiotic resistance genes in blood culture isolates. Each column represents a blood culture isolate from a single patient. The rows correspond to specific antibiotic resistance genes and are organized based on the antibiotic class to which the gene confers resistance. The color of each cell corresponds to the presence or absence of an antibiotic resistance gene within the blood culture isolate. FQ, fluoroquinolones; TCN, tetracyclines; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

plausible that the gut can serve as a reservoir for typically non-enteric pathogens in HSCT recipients, it is likely that sampling of other body sites will be needed to predict many BSI episodes caused by these organisms.

The detection of antibiotic resistance genes predicted phenotypic resistance to several classes of antibiotics. As expected, we identified the *vanA* gene complex in both VRE isolates and several classes of ESBL genes in Enterobacteriaceae isolates with resistance to third-generation cephalosporins. These results support the utility of rapid molecular testing of positive blood cultures for the *vanA* and ESBL genes, as was previously described and is currently used in many clinical settings [32-34]. Reliably predicting phenotypic resistance to other classes of antibiotics with rapid molecular assays has proven more challenging. In our cohort, the detection of aminoglycoside, fluoroquinolone, tetracycline, and trimethoprim-sulfamethoxazole resistance genes correlated poorly with phenotypic resistance to these antibiotics, possibly reflecting the complex mechanisms that often account for resistance to these antibiotic classes in Enterobacteriaceae [35]. For instance, although the most common mechanism for high-level resistance to fluoroquinolones is through mutations in the genes that encode type II topoisomerases (eg, *gyrA, gyrB, parC*), fluoroquinolone susceptibility can vary considerably even among strains with identical mutations in these genes, indicating the importance of other resistance mechanisms [36,37].

This study has several limitations. First, it included a relatively small number of patients, and the majority of BSI episodes were caused by *E. faecium* or *E. coli*. Although these species are among the most frequently detected pathogens in HSCT recipients, whether the dynamics of gut colonization that preceded these BSI episodes would apply to other bacterial species or fungi is unclear. In addition, the patterns of pathogen colonization before BSI onset that we describe are based on relative abundances that are necessarily correlated with the relative abundances of other gut microbes, and do not reflect absolute abundances. Moreover, although temporal associations between antibiotic exposures and rising abundances of the BSI strains in the gut microbiota were frequently seen, other factors may have contributed in this medically complex patient population. Our analyses were limited to children who developed a BSI episode, and it is likely that the observed patterns of pathogen colonization also occur in children who do not go on to develop BSI. We compared *E. faecium* and *E. coli* strains in fecal samples and blood at an unprecedented number of core genomic loci and applied a stringent definition of clonality, but there are no established cutoffs to definitively determine whether 2 strains of a bacterial species are genetically related. Finally, we were unable to compare the genetic similarity of the fecal strain to the blood culture isolate for 2 BSI episodes (patients 1 and 4), because too few reads were obtained for the BSI species in the gut microbiota.

In summary, we provide the most conclusive genomic data reported to date supporting a gastrointestinal source for BSI pathogens in HSCT recipients. High or rising relative abundances of bacterial strains in the gut microbiota preceded most BSI episodes in this small cohort. These data provide a basis for future studies evaluating the utility of serial whole-genome sequencing of the gut microbiota and other

potential pathogen reservoirs to identify HSCT recipients who are high risk for BSI.

## DECLARATION OF COMPETING INTEREST

There are no conflicts of interest to report.

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## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.07.019.

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