

Article

Pre-Transplant Antibiotic Exposures and Intestinal Microbiome Diversity in Allo-HSCT Recipients: A Prospective Cohort Study

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Abstract

Intestinal microbiome dysbiosis has been associated with transplant-related mortality and graft-versus-host disease in allo-HSCT patients. We assessed how pre-transplant antibiotic and antineoplastic exposures, together with multidrug-resistant colonization, are associated with baseline gut microbiome diversity at allo-HSCT. We conducted a prospective, single-center cohort study at Fundeni Clinical Institute (Bucharest, Romania) between August 2024 and June 2025, enrolling 52 allo-HSCT recipients and 27 healthy controls. Fecal samples were collected before conditioning. Gut microbiome composition was assessed via 16S rRNA gene sequencing and analyzed using QIIME2 and R. Associations were evaluated using Wilcoxon test, multivariable linear regression, and PERMANOVA. Shannon diversity was significantly lower in patients (median 4.71, IQR 3.97–5.44) than in healthy controls (median 6.09, IQR 5.87–6.28; $p < 0.001$). In bivariate analyses, carbapenem (p adj = 0.02) and oxazolidinone exposure (p adj = 0.005) were associated with reduced diversity, while immunotherapy was associated with higher diversity (p adj = 0.042). Broad-spectrum penicillin (p adj = 0.062) and ESBL colonization (p adj = 0.066) did not reach significance. In the multivariable antibiotic model, although the overall model was statistically significant (model $p = 0.039$), no individual antibiotic class remained significantly associated with Shannon diversity after adjustment for co-exposures. Beta diversity differed modestly with carbapenem exposure ($R^2 = 0.033$, $p = 0.019$). Pre-transplant antibiotic exposures were associated with lower gut microbiome diversity at allo-HSCT admission, with patterns consistent with a cumulative rather than a class-specific association. These findings support antibiotic stewardship in pre-transplant care.



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1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for a broad spectrum of hematological malignancies. However, it carries substantial morbidity driven by infectious complications, graft-versus-host disease (GVHD),

and transplant-related mortality [1]. The intestinal microbiome is crucial for maintaining immune balance, resisting colonization, and keeping the intestinal barrier intact. The gut harbours a densely populated and metabolically active microbial community that contributes to host physiology through several vital functions: it ferments dietary fibre into short-chain fatty acids that nourish the colonic epithelium and exert anti-inflammatory effects, it primes and regulates both innate and adaptive immune responses, and it confers colonization resistance against invading pathogens by competing for nutrients and ecological niches. The integrity of these functions depends on a diverse and balanced microbial ecosystem, so that its disruption (dysbiosis) has direct consequences for mucosal barrier function and immune homeostasis. The state of this ecosystem is commonly summarized using two complementary ecological measures. Alpha diversity describes the diversity within an individual sample, reflecting both the number of distinct taxa present (richness) and how evenly their abundances are distributed (evenness); the Shannon index, used in the present study, integrates both components. Beta diversity, by contrast, quantifies the difference in microbial community composition between samples, capturing how distinct two communities are from one another; in this study it was assessed using the Bray–Curtis dissimilarity. Its disruption during allo-HSCT is connected to negative clinical outcomes [2]. Landmark multicenter studies have demonstrated that lower intestinal microbial diversity at engraftment independently predicts transplant-related mortality, establishing the microbiome as a clinically relevant biomarker in this setting [3].

Importantly, microbiome disruption is not limited to the post-conditioning period. Serial sampling studies have shown that the pre-conditioning microbiome at HSCT admission may differ substantially in composition from the patient's original baseline before chemotherapy, reflecting incomplete ecological recovery from prior treatment-related perturbations [4]. Among these treatments, broad-spectrum antibiotics, especially carbapenems and cephalosporins, can disrupt the microbiome significantly by reducing key obligate anaerobic populations and promoting the growth of antibiotic-resistant pathogens [5].

The rising occurrence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales colonization in hematological patients complicates the situation. ESBL colonization at hospital admission shows previous antibiotic pressure and can affect the intestinal microbial ecosystem. However, its specific effect on microbiome diversity at the time of allo-HSCT admission has not been thoroughly studied [6].

Similarly, the contribution of antineoplastic agents to baseline microbiome disruption remains incompletely characterized. While cytotoxic chemotherapy is known to alter intestinal epithelial integrity and indirectly affect microbial composition [7], and recent work has linked baseline gut microbiome features to hematologic recovery in AML patients receiving induction therapy [8], the differential effects of specific drug classes—including hypomethylating agents, venetoclax, and immunotherapies—on microbiome diversity at the time of transplant admission have not been systematically evaluated.

In this prospective cohort study, we aimed to understand how pre-transplant antibiotics, cancer treatments, and multidrug-resistant bacterial colonization affect the microbiome diversity at the time of admission for allo-HSCT. The aim of this study was to identify pre-transplant factors associated with baseline gut microbiome diversity at allo-HSCT admission, rather than to establish a causal effect of any individual exposure. Given the observational design and the modest cohort size, the analysis is explicitly exploratory and hypothesis-generating in nature.

2. Materials and Methods

2.1. Study Design and Population

We conducted a prospective, single-center observational cohort study at Fundeni Clinical Institute, Bucharest, Romania. Adult patients (≥ 18 years) consecutively admitted for allogeneic hematopoietic stem cell transplantation between August 2024 and June 2025 were eligible for enrollment, regardless of primary diagnosis, donor type, conditioning intensity, or GVHD prophylaxis regimen. Patients were excluded if they were unable or unwilling to provide written informed consent, had a pre-existing diagnosis of inflammatory bowel disease, or were unable to provide a fecal sample at the time of admission. A total of 56 patients were enrolled, of whom 52 were included in the final analysis; 4 patients were excluded due to failed DNA extraction from the fecal sample, which precluded 16S rRNA sequencing.

Donor type was classified according to high-resolution HLA matching at HLA-A, -B, -C, -DRB1, and -DQB1 loci: matched unrelated donors (MUD) were defined as 10/10 matched; mismatched unrelated donors (MMUD) had a single allele mismatch (9/10); matched related donors (MRD) were 10/10 HLA-identical siblings; haploidentical donors (HAPLO) were related donors sharing one HLA haplotype.

Twenty-seven healthy adult volunteers with no history of hematological, gastrointestinal, metabolic, or autoimmune disease were recruited as controls. Healthy controls had received no antibiotics, probiotics, prebiotics, or any other supplements or medications capable of influencing intestinal flora within the six months preceding sample collection. The healthy control group had a median age of 35.0 years (IQR 31.0–43.0; range 18–57) and comprised 18 men (66.7%) and 9 women (33.3%). None of the volunteers were employed in a hospital or any healthcare-related occupation, none were healthcare workers, and none had been hospitalized in the six months preceding sample collection, minimizing the likelihood of exposure to the hospital microbial environment or to nosocomial flora.

2.2. Fecal Sample Collection

Fecal samples were collected longitudinally at five pre-specified time points: at hospital admission, before starting conditioning therapy (T0); on the day of transplantation (T1); 30 days after transplantation (T2); 100 days after transplantation (T3); and at the onset of biopsy-proven gastrointestinal acute graft-versus-host disease (Tx), when applicable. The present analysis focuses exclusively on the T0 sample, which captures the baseline intestinal microbiome at the time of transplant admission, reflecting the combined effects of prior treatments, antibiotic use, and colonization status, without any influence from conditioning or other transplant-related procedures. Healthy controls provided a single fecal sample at enrollment. All participants collected approximately 1–2 g of stool in sterile containers. Samples were immediately frozen at -20°C , transferred to the laboratory within 24 h, and stored at -80°C until DNA extraction.

2.3. DNA Extraction and 16S rRNA Gene Sequencing

Total genomic DNA was extracted from fecal samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentration and purity were assessed by NanoDrop spectrophotometry (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit fluorometry (Thermo Fisher Scientific, Waltham, MA, USA).

The V4–V5 hypervariable region of the bacterial 16S rRNA gene was amplified using the 515F and 944R primers, as previously described [9]. Library amplification was performed in a 20 μL reaction volume using KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland), containing 20 ng of template DNA (diluted in 5 μL TE buffer), 0.3 pmol

(1 µL) of each primer, 10 µL of KAPA Master Mix, and 3 µL of molecular biology grade water. Thermal cycling consisted of an initial denaturation at 95 °C for 3 min, followed by 20 cycles of 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min 30 s, with a final elongation at 72 °C for 5 min. Post-PCR cleanup and size selection were performed using the HighPrep PCR-DX system (MagBio Genomics, Gaithersburg, MD, USA) according to the manufacturer's protocol.

Indexing PCR was performed using the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). One µL of purified amplicon was added to a master mix containing 10 µL of KAPA Master Mix, 1 µL of i7 index, 1 µL of i5 index, and 7 µL of water. Amplification conditions were: initial denaturation at 95 °C for 3 min, followed by 12 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s, with a final elongation at 72 °C for 5 min. A second round of HighPrep PCR-DX purification was performed.

Sequencing was performed on an Illumina MiSeqDx platform (Illumina, San Diego, CA, USA) using v3 chemistry with 2 × 300 bp paired-end reads. Negative extraction controls were included throughout the workflow to monitor for contamination. Raw sequencing reads were demultiplexed using the Casava software v1.8.2 package (Illumina, San Diego, CA, USA).

2.4. Bioinformatic Analysis

Raw sequencing reads were processed using QIIME2 v2024.10 [10]. Quality filtering, denoising, and chimera removal were performed using DADA2 (q2-dada2 plugin, v2024.10) [11], generating amplicon sequence variants (ASVs) at single-nucleotide resolution. Taxonomic classification was assigned using a Naive Bayes classifier trained on the V4–V5 region of the Greengenes (version 13_8) reference database, with a confidence threshold of 0.70. The qiime feature-table summarize function was used to visualize sample counts and, together with the qiime diversity alpha-rarefaction curve, used to choose a count that maximized the number of samples retained for analysis, while still reaching a plateau in alpha diversity. All diversity metrics were computed using the qiime diversity core-metrics-phylogenetic function on the rarefied feature table, with sequencing depth 1577. This depth was selected to retain all evaluable samples in the analysis while ensuring that rarefaction curves had reached a plateau. This rarefaction depth therefore represents a deliberate balance between sequencing depth and sample retention: selecting a higher threshold would have excluded the samples with the lowest read counts and reduced the already modest cohort, whereas the chosen depth retained the full set of evaluable samples at a point where the alpha-rarefaction curves had already plateaued, indicating that the dominant diversity signal had been adequately captured. Because alpha- and beta-diversity metrics are computed on amplicon sequence variants generated by DADA2 prior to taxonomic assignment, these community-level measures (Shannon diversity and Bray–Curtis dissimilarity) are largely independent of the particular taxonomic reference database used, as they reflect the relative abundance structure of the community rather than fine-grained taxonomic labelling. Alpha diversity at T0 was quantified using the Shannon diversity, reflecting both species richness and evenness. Beta diversity was assessed using Bray–Curtis dissimilarity.

2.5. Statistical Analysis

Continuous variables were reported as medians and interquartile ranges (IQRs). Categorical variables were expressed as frequencies and percentages. The Wilcoxon rank-sum test was used to assess differences in Shannon diversity between patients with and without specific pre-transplant exposures. These raw *p*-values were added to boxplots (created using the package ggplot2 (4.0.1)), using the function stat_compare_means from the gg-

pubr (0.6.2) package. Benjamini–Hochberg adjusted p -values were calculated using the $p.adjust$ function in R to control the false discovery rate (FDR). FDR correction was applied separately within each exposure category: antibiotic classes (four tests: carbapenems, broad-spectrum penicillin, aminoglycosides, oxazolidinones), antineoplastic treatments (four tests: cytotoxic chemotherapy, hypomethylating agents, venetoclax, immunological therapies), and microbial colonization status (ESBL). Beta diversity was visualized using principal coordinates analysis (PCoA) conducted with the PCoA function from the ape package (5.8-1). We evaluated differences in microbial community composition between exposure groups using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations, using the $adonis2$ function from the vegan package (2.7-2) [12]. Homogeneity of multivariate dispersion was assessed using the $betadisper$ [13] and $permutest$ functions from the vegan package (2.7-2), prior to interpreting PERMANOVA results. Multivariable linear regression models were fitted using the lm function in base R to assess the independent associations of pre-transplant exposures with Shannon diversity at T0, with separate models for antibiotic classes and antineoplastic treatments. Multicollinearity among the independent variables in each multivariable model was assessed using the variance inflation factor (VIF), computed with the vif function from the car package; a VIF below 5 was considered to indicate the absence of problematic collinearity. Given the modest cohort size ($n = 52$), each multivariable model was deliberately restricted to the exposure variables of interest within a single category, in order to maintain an adequate ratio of observations to estimated parameters and to avoid model overfitting; additional clinical covariates were therefore not incorporated into these models. All statistical analyses were performed and visualized using R version 4.5.2. (R Foundation for Statistical Computing, Vienna, Austria).

2.6. Clinical Data Collection

Pre-transplant antibiotic exposures within 3 months of admission were recorded for each patient, including carbapenems, cephalosporins, broad-spectrum penicillin (piperacillin-tazobactam), aminoglycosides, polymyxins, and oxazolidinones. For antineoplastic treatments, data collected include the drugs received before admission, classified as cytotoxic chemotherapy, hypomethylating agents, venetoclax, and immunotherapies (including monoclonal antibodies and molecularly targeted agents), all collected within 6 months before admission.

The asymmetric exposure windows (three months for antibiotics, six months for antineoplastic agents) were chosen to reflect the differential pharmacokinetic and biological persistence of these treatment categories on the gut microbiome. Antibiotics usually have quick but short-term effects on the microbiome. In contrast, cancer treatment regimens are given in cycles over several months. They need a longer period to measure the total exposure during both the induction and consolidation phases. Exposures were recorded as binary variables (exposed versus not exposed within the predefined window) for each drug class. Granular pharmacological parameters such as cumulative duration of therapy, total dose, and the exact interval between the last administration and sample collection were not analyzed, and exposure was therefore modelled at the class level only.

Microbial carriage status at admission was determined through routine rectal swab screening. Colonization for ESBL, CRE, and VRE was defined as the presence of extended-spectrum beta-lactamase-producing Enterobacterales, carbapenem-resistant Enterobacterales, and vancomycin-resistant Enterococci, respectively.

3. Results

3.1. Study Population

A total of 52 adult patients admitted for allo-HSCT and 27 healthy controls were included in the final analysis. Baseline patient characteristics are summarized in Table 1. The median age at transplant admission was 44.8 years (IQR 36.0–51.3), and 31 patients (59.6%) were male. The 27 healthy controls had a median age of 35.0 years (IQR 31.0–43.0) and comprised 18 men (66.7%) and 9 women (33.3%); controls were therefore broadly comparable to patients in sex distribution and somewhat younger, as expected for a healthy volunteer group. The most frequent diagnosis was acute myeloid leukemia (AML) in 29 patients (55.8%), followed by acute lymphoblastic leukemia (ALL) in 10 (19.2%), chronic myeloid leukemia (CML) in 6 (11.5%), and other hematological malignancies in 7 (13.5%).

Table 1. Baseline characteristics of patients undergoing allo-HSCT (n = 52).

Characteristic	n (%)
Patient demographics	
Sex, male	31 (59.6%)
Primary diagnosis	
Acute myeloid leukemia (AML)	29 (55.8%)
Acute lymphoblastic leukemia (ALL)	10 (19.2%)
Chronic myeloid leukemia (CML)	6 (11.5%)
Other hematological malignancies	7 (13.5%)
Donor characteristics	
Matched unrelated donor (MUD)	18 (34.6%)
Mismatched unrelated donor (MMUD)	16 (30.8%)
Matched related donor (MRD)	9 (17.3%)
Haploidentical donor (HAPLO)	9 (17.3%)
Graft source	
Peripheral blood stem cells (PBSC)	52 (100.0%)
Conditioning	
Non-myeloablative conditioning (NMA)	1 (1.9%)
Reduced-intensity conditioning (RIC)	26 (50.0%)
Reduced-toxicity conditioning (RTC)	21 (40.4%)
Myeloablative conditioning (MAC)	4 (7.7%)
Immunosuppression	
PTCy + MMF + CNI prophylaxis	49 (94.2%)
MTX + CNI prophylaxis	2 (3.8%)
ATG + MTX + CNI prophylaxis	1 (1.9%)
HCT-CI score	
HCT-CI low risk	30 (57.7%)
HCT-CI intermediate risk	18 (34.6%)
HCT-CI high risk	4 (7.7%)

HCT-CI = Hematopoietic Cell Transplantation-specific Comorbidity Index; PTCy = post-transplant cyclophosphamide; MMF = mycophenolate mofetil; CNI = calcineurin inhibitor; MTX = methotrexate; ATG = anti-thymocyte globulin.

Regarding donor type, the majority of patients received grafts from unrelated donors: 18 patients (34.6%) from a matched unrelated donor (MUD) and 16 patients (30.8%) from a mismatched unrelated donor (MMUD). A matched related donor (MRD) was used in 9 patients (17.3%), and an equal number (9 patients, 17.3%) underwent haploidentical

transplantation (HAPLO). Conditioning regimens were predominantly of reduced intensity: 26 patients (50.0%) received reduced-intensity conditioning (RIC), and 21 patients (40.4%) received reduced-toxicity conditioning (RTC), while myeloablative conditioning (MAC) was administered in 4 patients (7.7%) and non-myeloablative conditioning (NMA) in only 1 patient (1.9%). All 52 patients received peripheral blood stem cells (PBSC) as the graft source; no bone marrow or umbilical cord blood transplants were included in this cohort (Table 1). Graft-versus-host disease (GVHD) prophylaxis consisted mainly of post-transplant cyclophosphamide combined with mycophenolate mofetil and a calcineurin inhibitor (PTCy + MMF + CNI), used in 49 patients (94.2%); the remaining patients received either MTX + CNI (2 patients, 3.8%) or ATG + MTX + CNI (1 patient, 1.9%).

According to the Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI), most patients were classified as low risk, with 30 patients (57.7%) having a score of 0. Intermediate risk (score 1–2) was observed in 18 patients (34.6%), while 4 patients (7.7%) were classified as high risk (score ≥ 3).

Pre-transplant exposures within three months prior to admission are summarized in Table 2. Regarding antibiotic exposure, 19 patients (36.5%) had received broad-spectrum penicillins, 13 (25.0%) aminoglycosides, 13 (25.0%) oxazolidinones, 9 (17.3%) carbapenems, 6 (11.5%) cephalosporins, and 2 (3.8%) polymyxins. Regarding antineoplastic treatments, 37 patients (71.2%) had received cytotoxic chemotherapy, 15 (28.8%) had received hypomethylating agents, 11 (21.2%) had received venetoclax, and 18 (34.6%) had received immunotherapies. ESBL colonization at admission was documented in 16 patients (30.8%), carbapenem-resistant Enterobacterales (CRE) colonization in 3 (5.8%), and vancomycin-resistant Enterococci (VRE) colonization in 6 (11.5%).

Table 2. Pre-transplant exposures and microbial colonization status at admission (n = 52).

Characteristic	Exposed n (%)
Pre-transplant antibiotic exposures (within 3 months of admission)	
Broad-spectrum penicillin	19 (36.5%)
Aminoglycosides	13 (25.0%)
Oxazolidinones	13 (25.0%)
Carbapenems	9 (17.3%)
Cephalosporins	6 (11.5%)
Polymyxins (Colistin)	2 (3.8%)
Pre-transplant antineoplastic treatments (within 6 months of admission)	
Cytotoxic chemotherapy	37 (71.2%)
Hypomethylating agents	15 (28.8%)
Venetoclax	11 (21.2%)
Immunological therapies	18 (34.6%)
Microbial carriage at admission	
ESBL colonization	16 (30.8%)
CRE colonization	3 (5.8%)
VRE colonization	6 (11.5%)

ESBL = extended-spectrum beta-lactamase-producing *Enterobacterales*; CRE = carbapenem-resistant *Enterobacterales*; VRE = vancomycin-resistant *Enterococci*.

Cephalosporins (n = 6, 11.5%) and polymyxins (n = 2, 3.8%) were not analyzed separately for associations with alpha or beta diversity due to the small number of exposed patients, which precluded reliable statistical inference. Similarly, CRE (n = 3, 5.8%) and VRE (n = 6, 11.5%) carriage status were not evaluated as separate exposure variables for the same reason; only ESBL colonization (n = 16, 30.8%) was analyzed in detail.

3.2. Association of Pre-Transplant Antibiotic Exposures with Alpha Diversity at Admission

Shannon diversity at T0 was significantly lower in patients who had received carbapenems within three months before admission than in those who had not. The median was 4.02 (IQR 3.81, 4.17), compared to 4.97 (IQR 3.96, 5.40), with p adj = 0.02 (Figure 1A), suggesting that prior exposure to carbapenems is associated with substantial disruption of the baseline microbiome at the time of transplant admission.

Similarly, patients with prior exposure to oxazolidinone agents showed significantly lower Shannon diversity at T0 than those without prior exposure. The median was 4.00 (IQR 2.94 to 4.21) in the exposed group, and 5.08 (IQR 4.19 to 5.40) in the unexposed group, with p adj = 0.005 (Figure 1B), the lowest p -value among all antibiotic classes evaluated.

Broad-spectrum penicillin exposure showed a trend toward lower Shannon diversity that did not reach statistical significance after FDR correction (p adj = 0.062), whereas aminoglycoside exposure showed no association with alpha diversity at admission (p adj = 0.20) (Figure 1C,D). Median Shannon values and pairwise comparisons for all antibiotic classes are summarized in Table 3.

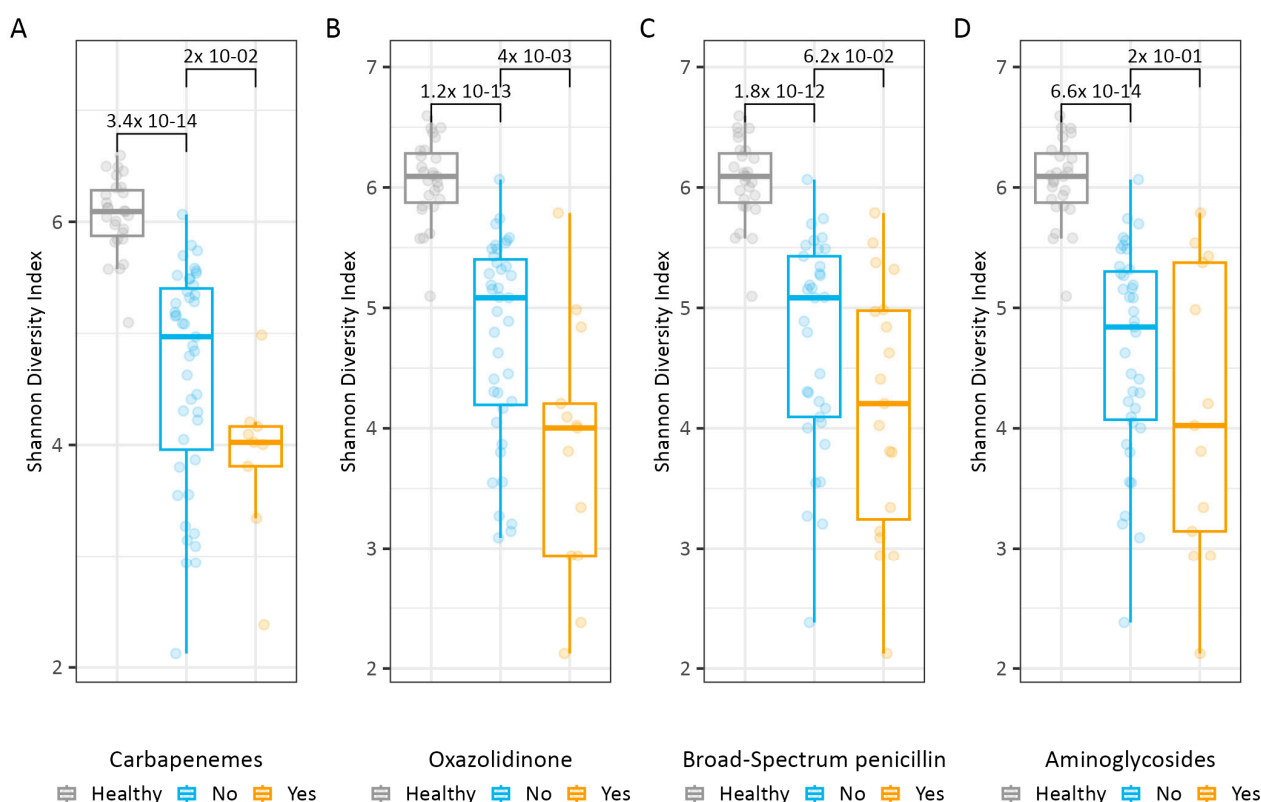


Figure 1. Shannon diversity index at T0 stratified by pre-transplant antibiotic exposures. (A) Carbapenems; (B) Oxazolidinones; (C) Broad-spectrum penicillin; (D) Aminoglycosides. Each panel compares exposed versus unexposed patients. Boxes represent the median and interquartile range, whiskers extend to 1.5× the interquartile range, and points denote individual patients. The p -values displayed on the plots are unadjusted (raw) Wilcoxon rank-sum p -values generated by `stat_compare_means`; the corresponding Benjamini–Hochberg FDR-adjusted p -values, which form the basis for the interpretation in the text and in Table 3, are reported separately in Table 3. Comparisons against the healthy control group and are presented in Table 3.

Table 3. Shannon diversity index at T0 stratified by pre-transplant exposure.

Variable	Group	n	Shannon Median (IQR)	<i>p</i> adj (No vs. Yes)
Reference: Healthy controls	Healthy	27	6.09 (5.87–6.28)	—
All patients vs. healthy	All patients	52	4.71 (3.97–5.44)	<0.001 †
Antibiotic exposures (within 3 months of admission)				
Carbapenems	No	43	4.97 (3.96–5.40)	0.02
	Yes	9	4.02 (3.81–4.17)	
Broad-spectrum penicillin	No	33	5.08 (4.09–5.43)	0.062
	Yes	19	4.21 (3.24–4.98)	
Aminoglycosides	No	39	4.84 (4.07–5.30)	0.20
	Yes	13	4.02 (3.14–5.38)	
Oxazolidinones	No	39	5.08 (4.19–5.40)	0.005
	Yes	13	4.00 (2.94–4.21)	
Antineoplastic treatments (within 6 months of admission)				
Cytotoxic chemotherapy	No	15	4.31 (3.68–5.53)	0.72
	Yes	37	4.80 (4.00–5.27)	
Hypomethylating agents	No	37	4.80 (3.87–5.32)	0.97
	Yes	15	4.45 (3.98–5.31)	
Venetoclax	No	41	4.63 (3.87–5.32)	0.69
	Yes	11	4.89 (4.01–5.41)	
Immunological therapies	No	34	4.26 (3.62–5.16)	0.042
	Yes	18	5.14 (4.32–5.56)	
ESBL colonization status				
ESBL colonization	Negative	36	5.03 (4.02–5.39)	0.066
	Positive	16	4.26 (3.29–4.69)	

IQR = interquartile range; *p* adj = FDR-adjusted *p*-value (Benjamini–Hochberg, applied separately within each exposure category). † Wilcoxon rank-sum test.

3.3. Association of Pre-Transplant Antineoplastic Treatments with Alpha Diversity at Admission

Patients who received immunological therapies, including monoclonal antibodies and molecularly targeted agents, before admission showed significantly higher Shannon diversity than those who did not. The median for the immunotherapy group was 5.14 (IQR 4.32–5.56) compared to 4.26 (IQR 3.62–5.16) for the other group, with *p* adj = 0.042 (Figure 2A).

Prior cytotoxic chemotherapy (*p* adj = 0.72), exposure to hypomethylating agents (*p* adj = 0.97), and venetoclax therapy (*p* adj = 0.69) did not show significant differences in Shannon diversity at T0. These findings suggest that these cancer treatments do not, independently, reduce baseline intestinal microbiome diversity beyond the effects of concurrent antibiotic use (Figure 2B–D). Median Shannon values for all cancer treatment categories are summarized in Table 3.

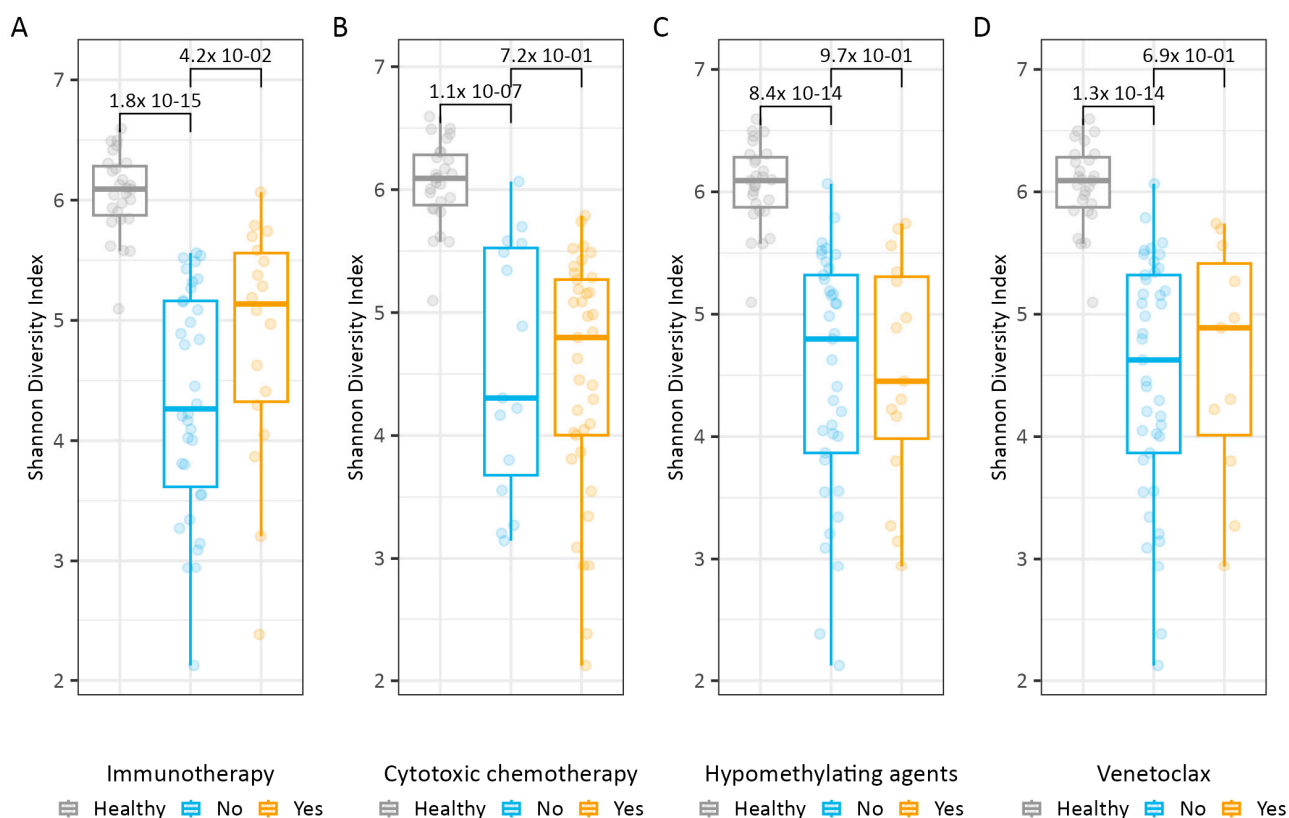


Figure 2. Shannon diversity index at T0 stratified by pre-transplant antineoplastic treatments. (A) Immunotherapy; (B) Cytotoxic chemotherapy; (C) Hypomethylating agents; (D) Venetoclax. Each panel compares exposed versus unexposed patients. Boxes represent the median and interquartile range, whiskers extend to $1.5 \times$ the interquartile range, and points denote individual patients. The p -values shown on the plots are unadjusted (raw) Wilcoxon rank-sum p -values; FDR-adjusted p -values are reported in Table 3.

3.4. Association of ESBL Colonization with Alpha and Beta Diversity at Admission

Patients with ESBL colonization at admission ($n = 16$, 30.8%) showed lower Shannon diversity compared to non-colonized patients (median 4.26 [IQR 3.29, 4.69] vs. 5.03 [IQR 4.02, 5.39]); however, this difference did not reach statistical significance after FDR correction ($p_{\text{adj}} = 0.066$) (Figure 3). The numerical direction of this difference is consistent with the hypothesis that ESBL colonization may reflect prior antibiotic pressure, but this association requires confirmation in larger cohorts.

Beta diversity analysis at T0 showed a statistically significant but modest difference in microbial community composition between patients with and without prior carbapenem exposure (Bray–Curtis PERMANOVA: $R^2 = 0.033$, $p = 0.019$), indicating that carbapenem exposure explains approximately 3.3% of the variance in community composition. A test for homogeneity of multivariate dispersion (betadisper) showed similar dispersion within each group ($F = 0.000244$, $p = 0.987$), indicating that the observed compositional differences are unlikely to be driven by within-group variation (Figure 4). On the other hand, the ESBL colonization status at admission did not show significant differences in beta diversity at T0 (PERMANOVA: $R^2 = 0.02$, $p = 0.377$; betadisper $p = 0.35$) (Figure 5).

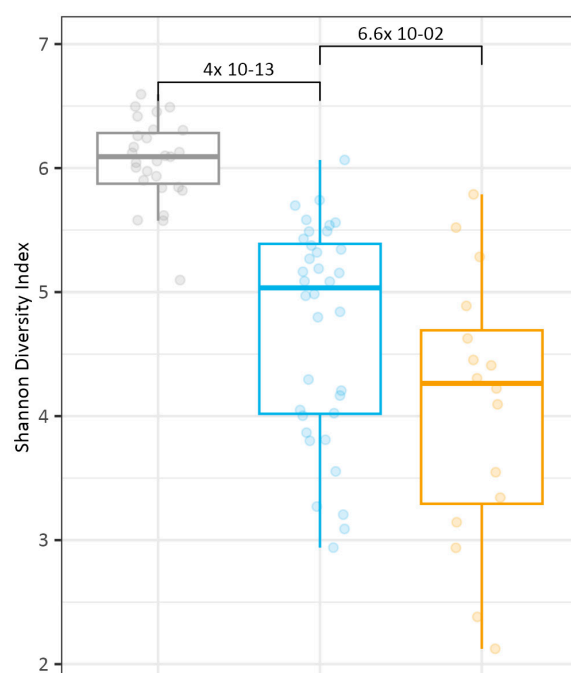


Figure 3. Shannon diversity index at T0 stratified by ESBL colonization status at admission. Boxes represent the median and interquartile range, whiskers extend to $1.5 \times$ the interquartile range, and points denote individual patients. The p -value shown is the unadjusted (raw) Wilcoxon rank-sum p -value; the FDR-adjusted value (p adj = 0.066) is reported in Table 3.

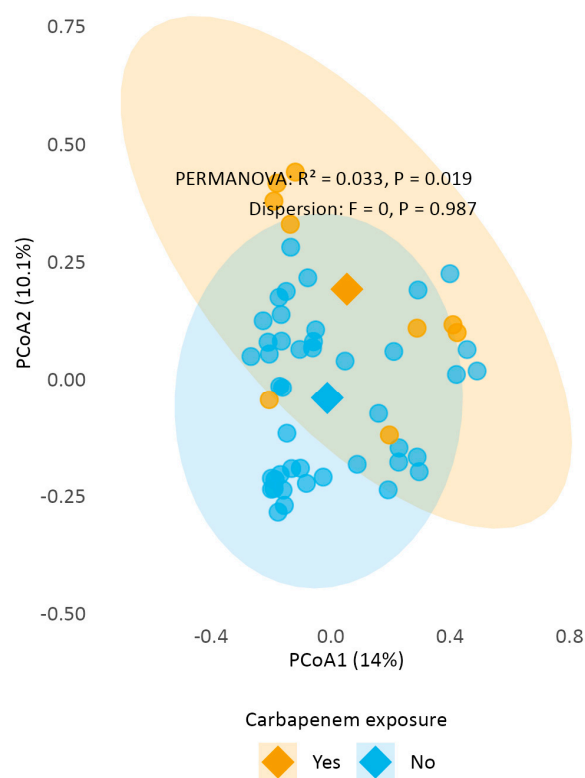


Figure 4. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarity at T0 stratified by carbapenem exposure. Each point represents one patient; axes show the first two principal coordinates with the percentage of variance explained. Group separation was tested by PERMANOVA ($R^2 = 0.033$, $p = 0.019$, 9999 permutations), with homogeneity of multivariate dispersion confirmed by betadisper prior to interpretation.

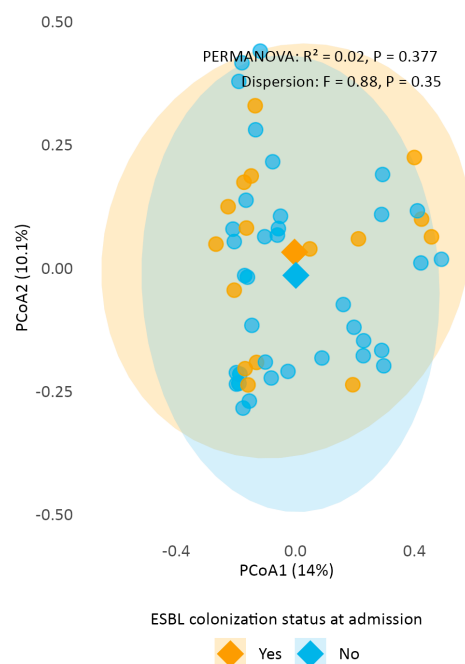


Figure 5. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarity at T0 stratified by ESBL colonization status at admission. Each point represents one patient; axes show the first two principal coordinates with the percentage of variance explained. Group separation was tested by PERMANOVA ($R^2 = 0.02$, $p = 0.377$, 9999 permutations; betadisper $p = 0.35$), indicating no significant difference in community composition by ESBL status.

3.5. Multivariable Analysis of Pre-Transplant Exposures

To assess whether individual antibiotic classes were independently associated with Shannon diversity at T0 after adjusting for co-exposures, we performed a multivariable linear regression model including carbapenems, broad-spectrum penicillins, aminoglycosides, and oxazolidinones as binary predictors. Cephalosporins were excluded due to the small number of exposed patients ($n = 6$). The overall model was statistically significant (adjusted $R^2 = 0.12$, $p = 0.039$); however, no individual antibiotic class retained statistical significance after adjustment for co-exposures (Table 4). The strongest signal was observed for oxazolidinones ($\beta = -0.87$, 95% CI -1.83 to 0.10 , $p = 0.078$), which approached but did not reach significance. A separate multivariable model including cytotoxic chemotherapy, hypomethylating agents, venetoclax, and immunological therapies was not statistically significant overall (adjusted $R^2 = -0.01$, $p = 0.505$), with no individual predictor reaching significance. Multicollinearity among the independent variables was low in both models, with all variance inflation factors well below the conventional threshold of 5. In the antibiotic model, VIF values were 1.94 for carbapenems, 1.92 for broad-spectrum penicillin, 2.17 for aminoglycosides, and 2.71 for oxazolidinones. In the antineoplastic model, VIF values were 1.42 for cytotoxic chemotherapy, 3.72 for hypomethylating agents, 3.04 for venetoclax, and 1.07 for immunological therapies. These results indicate that the regression estimates were not appreciably affected by collinearity among co-exposures.

Table 4. Multivariable linear regression of pre-transplant exposures and Shannon diversity at T0.

Variable	β	95% CI	<i>p</i> -Value
Antibiotic exposures model (adjusted $R^2 = 0.12$, $p = 0.039$)			
Carbapenems	−0.10	−1.40 to 0.83	0.82
Broad-spectrum penicillin	−0.29	−1.02 to 0.45	0.44
Aminoglycosides	0.24	−0.63 to 1.11	0.58
Oxazolidinones	−0.87	−1.83 to 0.10	0.078
Antineoplastic treatments model (adjusted $R^2 = -0.01$, $p = 0.505$)			
Cytotoxic chemotherapy	−0.02	−0.74 to 0.70	0.96
Hypomethylating agents	−0.04	−1.20 to 1.13	0.95
Venetoclax	0.19	−0.97 to 1.36	0.74
Immunological therapies	0.51	−0.09 to 1.11	0.091

Reference category for each binary predictor: not exposed. Cephalosporins ($n = 6$) and polymyxins ($n = 2$) were excluded from the antibiotic model due to insufficient sample size. CI = confidence interval.

4. Discussion

This study provides an exploratory characterization of the intestinal microbiome at the time of admission for allo-HSCT in a homogeneously studied prospective cohort of patients with hematological malignancies. Our findings confirm that significant intestinal dysbiosis is already present before initiation of conditioning, with substantially lower Shannon diversity in patients compared with healthy controls. The main contribution of this study is identifying specific pre-transplant factors that lead to changes in the baseline microbiome. The use of carbapenem and oxazolidinone stands out as the most consistent link among the antibiotic exposures examined in the bivariate analyses.

Our finding of lower Shannon diversity in patients exposed to carbapenems (median 4.02 vs. 4.97, p adj = 0.02) confirms previous research showing that carbapenems have significant effects on the gut microbiome [14–16]. The differences in beta diversity (PERMANOVA $R^2 = 0.033$, $p = 0.019$) are modest in size, explaining only 3.3% of the variance. However, they suggest that exposure to carbapenems is linked to more extensive changes in composition, not just a simple decrease in alpha diversity. Importantly, the consistency of dispersion was maintained between the exposure groups. This supports the idea that these compositional differences are not likely caused solely by differences within the groups. These observations are consistent with prior reports in HSCT populations: receipt of anaerobe-active agents such as piperacillin-tazobactam and carbapenems has been associated with reduced bacterial diversity and depletion of obligate anaerobes including Bifidobacteriales and Clostridiales [17], and a serial analysis of HSCT recipients found that broad-spectrum antibiotics failed to spare commensal populations, with no clearly commensal-sparing agent identified among those in routine use [18].

The strong association observed for oxazolidinones, primarily linezolid, is of interest, as this drug class targets Gram-positive organisms; broad-spectrum antibiotic exposure has been reviewed as a major driver of microbiome disruption in allo-HSCT recipients [19,20]. The magnitude of this effect (median Shannon 4.00 vs. 5.08, p adj = 0.005) warrants attention, although this association may reflect indirect ecological effects on commensal gram-positive populations or residual confounding by concurrent broad-spectrum antibiotic exposure or underlying severe infection [19]. A recent analysis of patients with acute myeloid leukemia undergoing induction chemotherapy reported a comparable signal, in which a greater number of oxazolidinone administrations was associated with a greater loss of microbial diversity, lending external support to the association observed in our cohort [21].

4.1. Multivariable Analysis and Overlapping Co-Exposures

In multivariable linear regression that included all four antibiotic classes, the overall model was significant (adjusted $R^2 = 0.12$, $p = 0.039$). This confirms that pre-transplant antibiotic exposure explains a meaningful portion of baseline microbiome variability. However, no individual antibiotic class remained significant after adjusting for co-exposures. This suggests that the relationships found for carbapenems and oxazolidinones may reflect the total antibiotic burden rather than effects specific to each class. This view aligns with patterns seen in other hematological groups. Patients receiving one broad-spectrum agent are typically exposed to multiple classes during induction or consolidation chemotherapy, making it methodologically challenging to isolate the contribution of any single class [5,20]. Comparable observations in surgical and ICU populations have shown that total antibiotic days, rather than the choice of any individual class, is the strongest predictor of microbiome disruption [22]. Consistent with this, a high-resolution metagenomic study of heavily antibiotic-treated HSCT recipients found that broad-spectrum β -lactam exposure in particular was associated with the most pronounced microbiome disruption, supporting the interpretation that cumulative broad-spectrum antibiotic pressure, rather than any single class in isolation, is the principal correlate of baseline dysbiosis [23]. Our findings should therefore be interpreted as supporting overall antibiotic stewardship in the pre-transplant period rather than implicating specific classes for selective avoidance.

The absence of a strong association between antineoplastic agents (cytotoxic chemotherapy, hypomethylating agents, venetoclax) and Shannon diversity at admission supports the idea that the intestinal microbiome can recover between cycles of antineoplastic therapy. In contrast, concurrent antibiotic use tends to have more lasting effects. The higher Shannon diversity seen in patients receiving immunotherapy (median 5.14 vs. 4.26, $p_{\text{adj}} = 0.042$) raises the idea that some immunomodulatory agents may have neutral or even positive effects on the microbiome. However, this finding should be seen as a starting point for further exploration, not as definitive proof. It likely reflects selection bias. Patients receiving immunotherapy may be a clinically different group, often with shorter treatment histories, lower overall antibiotic exposure, or better general health. Disentangling a true biological effect of immunotherapy on the gut microbiome from confounding by indication will require larger cohorts with formal adjustment for treatment intensity and timing.

Regarding ESBL colonization, the lower Shannon diversity numerically observed in colonized patients (median 4.26 vs. 5.03, $p_{\text{adj}} = 0.066$) did not reach statistical significance after FDR correction. While the direction of this difference is consistent with the hypothesis that ESBL carriage may serve as a marker of cumulative antibiotic pressure, our data are insufficient to support firm conclusions about a causal relationship in either direction. This interpretation is supported by the finding that beta diversity was not significantly altered by ESBL status, indicating that the underlying microbial community structure remains relatively preserved in colonized patients despite reduced diversity. This pattern is in line with a large matched case-control study in which ESBL-producing Enterobacterales carriage was not associated with significant differences in either alpha or beta diversity after multiple-testing correction [24]. Likewise, a prospective study of ESBL colonization in allo-HSCT recipients reported that colonization status was reflected in shifts in the relative abundance of specific taxa rather than in a marked change in overall community structure [25], consistent with our observation of reduced diversity without a corresponding beta-diversity shift.

4.2. Limitations

Several limitations should be acknowledged. First, this is a single-center study with a relatively modest sample size, which limits generalizability and statistical power. With

52 evaluable patients, the cohort was adequately powered to detect moderate-to-large differences in Shannon diversity for exposures with sufficient subgroup representation, but was underpowered to detect smaller, potentially clinically meaningful differences for less common exposures. The unbalanced sample sizes between exposed and unexposed subgroups (e.g., carbapenems 9 vs. 43; ESBL 16 vs. 36) reduce the precision of estimates and increase the likelihood of type II errors for borderline associations. Furthermore, the PERMANOVA result for carbapenem exposure ($R^2 = 0.033$) should be interpreted with caution given the unbalanced group sizes; although the betadisper test confirmed homogeneity of multivariate dispersion, the modest variance explained suggests that the observed compositional shift, while statistically significant, represents a relatively small fraction of overall microbiome variability. Larger multicenter cohorts will be needed to confirm both the reported associations and to detect more subtle effects. Given these constraints, the associations reported for exposures with small exposed subgroups (such as carbapenems and oxazolidinones) should be regarded as exploratory and hypothesis-generating, and the corresponding estimates should not be over-interpreted. In addition, because of the modest cohort size, the multivariable models were intentionally limited to the exposures of interest and were not further adjusted for clinical confounders such as age, primary diagnosis, prior hospitalizations, or treatment intensity; such adjustment would have risked overfitting at the available sample size and would require larger, adequately powered cohorts.

Second, the rarefaction depth used in our analysis (1577 reads per sample) was selected to retain all evaluable samples and ensure that rarefaction curves had reached a plateau, but this depth is lower than the higher thresholds (often >5000 reads) employed in some larger microbiome studies [3]. Although this depth is sufficient for robust estimation of Shannon diversity—which is more sensitive to dominant taxa than to rare community members—it may have limited the resolution of rare taxa detection and the granularity of beta diversity analyses. Taxonomic classification relied on the Greengenes reference database, which is less recently updated than alternatives such as SILVA or GTDB; while this is unlikely to have materially affected the community-level diversity metrics reported here, since these are computed on amplicon sequence variants prior to taxonomic assignment, it may have constrained the precision of fine-grained taxonomic labelling.

Third, although our cohort included patients with diverse hematological malignancies (AML, ALL, CML, and other diagnoses), each disease subgroup carries distinct treatment trajectories and antibiotic exposure patterns, which may differentially shape the gut microbiome [7,26,27]. We did not stratify analyses by primary diagnosis due to limited statistical power within subgroups. Future studies in larger, diagnosis-stratified cohorts will be needed to determine whether the associations observed here are consistent across hematological diseases.

The reliance on rectal swab screening for ESBL detection may underestimate true colonization rates compared to selective stool culture or molecular methods. Despite these limitations, the prospective design, comprehensive clinical phenotyping, and standardized sample collection protocol strengthen the validity of our findings.

4.3. Clinical Implications

Our findings suggest several important points for clinical practice. The observed association between certain antibiotic use and lower microbiome diversity at transplant admission emphasizes the need for stricter antibiotic management before transplant. Broad-spectrum antibiotics, such as carbapenems, should be used only for confirmed multidrug-resistant infections or in critical situations where alternatives are unavailable. Since the signals for individual antibiotic classes lost significance in the multivariable analysis,

stewardship efforts should aim to reduce overall antibiotic exposure rather than avoid specific classes. Regularly collecting microbiome data at transplant admission, along with detailed histories of antibiotic use, could help identify patients at higher risk for negative outcomes and inform tailored supportive care approaches.

5. Conclusions

This exploratory prospective cohort study found that pre-transplant antibiotic exposures, particularly carbapenems and oxazolidinones, were associated with lower intestinal microbiome diversity at the time of allo-HSCT admission in bivariate analyses. No individual antibiotic class remained significantly associated with diversity in the multivariable analysis after adjustment for co-exposures; while the overall antibiotic model was statistically significant, our data do not demonstrate an independent effect of any single class, and the findings are most consistent with cumulative antibiotic exposure rather than any one class being the relevant correlate of baseline microbiome diversity. These associations support antibiotic stewardship programs targeting reduction in overall broad-spectrum antibiotic burden in patients with hematological malignancies and warrant confirmation in larger, multicenter cohorts.

The higher Shannon diversity observed in patients who had received immunotherapy was an exploratory association that may reflect selection or confounding by indication rather than a biological effect, and should be interpreted with caution pending confirmation in larger, multicenter cohorts. The absence of a significant difference in beta diversity by ESBL colonization status, despite numerically lower Shannon diversity, suggests that culture-based colonization screening and community-level compositional analyses capture related but non-identical aspects of microbiome status. Overall, these findings should be understood as associations rather than evidence of causation and provide a basis for hypothesis-driven, adequately powered studies in this setting.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Fundeni Clinical Institute (approval number 18881, dated 17 April 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The 16S rRNA gene sequencing data and associated metadata generated and analyzed during this study are not publicly deposited at the time of submission due to ongoing patient privacy and institutional data-sharing review. The data are available from the corresponding author upon reasonable request, subject to approval by the Ethics Committee of Fundeni Clinical Institute.

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Abbreviations

The following abbreviations are used in this manuscript:

ALL	Acute lymphoblastic leukemia
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
AML	Acute myeloid leukemia
ATG	anti-thymocyte globulin
CML	Chronic myeloid leukemia
CNI	Calcineurin inhibitor
CRE	Carbapenem-resistant Enterobacterales
ESBL	Extended-spectrum beta-lactamase
GVHD	Graft-versus-host disease
HAPLO	Haploidentical donors
HCT-CI	Hematopoietic Cell Transplantation-specific Comorbidity Index
IQRs	Interquartile ranges
MAC	Myeloablative conditioning
MMF	Mycophenolate mofetil
MMUD	Mismatched unrelated donors
MRD	Matched related donors
MUD	Matched unrelated donors
NMA	Non-myeloablative conditioning
PBSC	Peripheral blood stem cells
PCoA	Principal coordinates analysis
PERMANOVA	Permutational multivariate analysis of variance
PTCy	Post-transplant cyclophosphamid
RIC	Reduced-intensity conditioning
RTC	Reduced-toxicity conditioning
VRE	Vancomycin-resistant Enterococci

References

1. Penack, O.; Marchetti, M.; Aljurf, M.; Arat, M.; Bonifazi, F.; Duarte, R.F.; Giebel, S.; Greinix, H.; Hazenberg, M.D.; Kröger, N.; et al. Prophylaxis and management of graft-versus-host disease after stem-cell transplantation for haematological malignancies: Updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol.* **2024**, *11*, e147–e159. [[CrossRef](#)] [[PubMed](#)]
2. Schluter, J.; Peled, J.U.; Taylor, B.P.; Markey, K.A.; Smith, M.; Taur, Y.; Niehus, R.; Staffas, A.; Dai, A.; Fontana, E.; et al. The gut microbiota is associated with immune cell dynamics in humans. *Nature* **2020**, *588*, 303–307. [[CrossRef](#)] [[PubMed](#)]
3. Peled, J.U.; Gomes, A.L.C.; Devlin, S.M.; Littmann, E.R.; Taur, Y.; Sung, A.D.; Weber, D.; Hashimoto, D.; Slingerland, A.E.; Slingerland, J.B.; et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N. Engl. J. Med.* **2020**, *382*, 822–834. [[CrossRef](#)] [[PubMed](#)]
4. Rashidi, A.; Kaiser, T.; Holtan, S.G.; Weisdorf, D.J.; Khoruts, A.; Staley, C. Pre-transplant recovery of microbiome diversity without recovery of the original microbiome. *Bone Marrow Transplant.* **2019**, *54*, 1115–1117. [[CrossRef](#)] [[PubMed](#)]
5. Modi, S.R.; Collins, J.J.; Relman, D.A. Antibiotics and the gut microbiota. *J. Clin. Investig.* **2014**, *124*, 4212–4218. [[CrossRef](#)] [[PubMed](#)]

6. Niyazi, D.; Vergiev, S.; Markovska, R.; Stoeva, T. Prevalence and molecular epidemiology of intestinal colonization by multidrug-resistant bacteria among hematopoietic stem-cell transplantation recipients: A Bulgarian single-center study. *Antibiotics* **2024**, *13*, 920. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Galloway-Peña, J.R.; Smith, D.P.; Sahasrabhojane, P.; Wadsworth, W.D.; Fellman, B.M.; Ajami, N.J.; Shpall, E.J.; Daver, N.; Guindani, M.; Petrosino, J.F.; et al. Characterization of oral and gut microbiome temporal variability in hospitalized cancer patients. *Genome Med.* **2017**, *9*, 21. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Salvestrini, V.; Conti, G.; D'Amico, F.; Cristiano, G.; Candela, M.; Cavo, M.; Turrone, S.; Curti, A. Gut microbiome as a potential marker of hematologic recovery following induction therapy in acute myeloid leukemia patients. *Cancer Med.* **2025**, *14*, e70501. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Abellan-Schneyder, I.; Matchado, M.S.; Reitmeier, S.; Sommer, A.; Sewald, Z.; Baumbach, J.; List, M.; Neuhaus, K. Primer, pipelines, parameters: Issues in 16S rRNA gene sequencing. *mSphere* **2021**, *6*, e01202-20. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [\[CrossRef\]](#)
13. Anderson, M.J. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **2006**, *62*, 245–253. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Fishbein, S.R.S.; Mahmud, B.; Dantas, G. Antibiotic perturbations to the gut microbiome. *Nat. Rev. Microbiol.* **2023**, *21*, 772–788. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Lange, K.; Buerger, M.; Stallmach, A.; Bruns, T. Effects of antibiotics on gut microbiota. *Dig. Dis.* **2016**, *34*, 260–268. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Galloway-Peña, J.R.; Shi, Y.; Peterson, C.B.; Sahasrabhojane, P.; Gopalakrishnan, V.; Brumlow, C.E.; Daver, N.G.; Alfayez, M.; Boddu, P.C.; Khan, M.A.W.; et al. Gut microbiome signatures are predictive of infectious risk following induction therapy for acute myeloid leukemia. *Clin. Infect. Dis.* **2020**, *71*, 63–71. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Tanaka, J.S.; Young, R.R.; Heston, S.M.; Jenkins, K.; Spees, L.P.; Sung, A.D.; Corbet, K.; Thompson, J.C.; Bohannon, L.; Martin, P.L.; et al. Anaerobic antibiotics and the risk of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* **2020**, *26*, 2053–2060. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Weber, D.; Hiergeist, A.; Weber, M.; Dettmer, K.; Wolff, D.; Hahn, J.; Herr, W.; Gessner, A.; Holler, E. Detrimental effect of broad-spectrum antibiotics on intestinal microbiome diversity in patients after allogeneic stem cell transplantation: Lack of commensal sparing antibiotics. *Clin. Infect. Dis.* **2019**, *68*, 1303–1310. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Nakagaki, M.; Hajkiewicz, K.; Roberts, J.A.; Henden, A.S. Cause and effect? A review of the impact of antibiotics on the gut microbiome in patients undergoing hematopoietic stem cell transplantation. *Expert Rev. Anti Infect. Ther.* **2025**, *23*, 863–876. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Taur, Y.; Xavier, J.B.; Lipuma, L.; Ubeda, C.; Goldberg, J.; Gobourne, A.; Lee, Y.J.; Dubin, K.A.; Socci, N.D.; Viale, A.; et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin. Infect. Dis.* **2012**, *55*, 905–914. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Franklin, S.; Ramont, C.; Batool, M.; McMahon, S.; Sahasrabhojane, P.; Blazier, J.C.; Kontoyannis, D.P.; Ni, Y.; Galloway-Peña, J. Characterization of antibiotic administration factors associated with microbiome disruption and subsequent antibiotic-resistant infection and colonization events in acute myeloid leukemia patients receiving chemotherapy. *Antibiotics* **2025**, *14*, 770. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Peto, L.; Fawcett, N.; Kamfose, M.M.; Scarborough, C.; Peniket, A.; Danby, R.; Peto, T.E.A.; Crook, D.W.; Llewelyn, M.J.; Walker, A.S. The impact of different antimicrobial exposures on the gut microbiome in the ARMORD observational study. *eLife* **2025**, *13*, RP97751. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Nørgaard, J.C.; Jørgensen, M.; Moestrup, K.S.; Ilett, E.E.; Zucco, A.G.; Marandi, R.Z.; Julian, M.N.; Paredes, R.; Lundgren, J.D.; Sengeløv, H.; et al. Impact of antibiotic treatment on the gut microbiome and its resistome in hematopoietic stem cell transplant recipients. *J. Infect. Dis.* **2023**, *228*, 28–36. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Boyd, A.; El Dani, M.; Ajrouche, R.; Demontant, V.; Cheval, J.; Lacombe, K.; Cosson, G.; Rodriguez, C.; Pawlotsky, J.M.; Woerther, P.L.; et al. Gut microbiome diversity and composition in individuals with and without extended-spectrum β -lactamase-producing Enterobacterales carriage: A matched case-control study in an infectious diseases department. *Clin. Microbiol. Infect.* **2024**, *30*, 1154–1163. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Corcione, S.; Ferrocino, I.; Lupia, T.; Busca, A.; Bianco, G.; Dellacasa, C.; Giaccone, L.; Brunello, L.; Butera, S.; Costa, C.; et al. Influence of ESBL colonization status on gut microbiota composition during allogeneic hematopoietic stem cell transplantation. *Sci. Rep.* **2025**, *15*, 1275. [\[CrossRef\]](#) [\[PubMed\]](#)

26. Galloway-Peña, J.R.; Smith, D.P.; Sahasrabhojane, P.; Ajami, N.J.; Wadsworth, W.D.; Daver, N.G.; Chemaly, R.F.; Marsh, L.; Ghantaji, S.S.; Pemmaraju, N.; et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. *Cancer* **2016**, *122*, 2186–2196. [[CrossRef](#)] [[PubMed](#)]
27. Yu, D.; Yu, X.; Ye, A.; Xu, C.; Li, X.; Geng, W.; Zhu, L. Profiling of gut microbial dysbiosis in adults with myeloid leukemia. *FEBS Open Bio* **2021**, *11*, 2050–2059. [[CrossRef](#)] [[PubMed](#)]

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