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Gut Microbiota Dysbiosis Following Allogeneic Bone Marrow Transplantation is Characterized by Proteobacteria Dominance and Reduced Microbial Diversity

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Abstract

Background: The gut microbiota plays a vital role in maintaining immune homeostasis, metabolic balance, and protection against pathogens. In patients undergoing allogeneic bone marrow transplantation (allo-BMT), factors such as chemotherapy, irradiation, antibiotic exposure, and immunosuppressive therapy disrupt the intestinal microbial ecosystem, leading to dysbiosis. This imbalance has been strongly associated with adverse clinical outcomes, including infections and graft-versus-host disease (GVHD).

Aim: This study aimed to investigate alterations in gut microbial composition and diversity in allo-BMT patients and evaluate their potential implications in transplant-related complications.

Methodology: A comparative study was conducted on allo-BMT recipients. Fecal samples were collected before and approximately 30 days after transplantation. Microbial DNA was extracted and analyzed using high-throughput next-generation sequencing targeting the 16S rRNA gene. Bioinformatic and statistical analyses were performed to assess taxonomic composition, alpha diversity, and microbial community structure.

Results: Post-transplant samples showed a significant shift in gut microbiota composition, with dominance of Proteobacteria (up to ~99%) and a marked reduction in Firmicutes and Bacteroidetes. There was enrichment of opportunistic pathogens such as *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Serratia rubidaea*, alongside depletion of beneficial commensals including *Prevotella copri*, *Streptococcus salivarius*, and *Lactobacillus salivarius*. Alpha diversity indices indicated a substantial decline in microbial richness and evenness, reflecting a transition to a dysbiotic, pathogen-dominated state.

Conclusion: Allo-BMT is associated with profound gut microbiota dysbiosis, which may increase susceptibility to infections and GVHD. These findings highlight the potential of microbiota profiling as a predictive biomarker and support the need for microbiota-targeted therapeutic strategies.

Keywords: Gut microbiota; Bone marrow transplantation; Dysbiosis; 16S rRNA; GVHD

Impact Statement

This study highlights the critical role of gut microbiota dysbiosis in shaping clinical outcomes following allogeneic hematopoietic stem cell transplantation. The observed loss of beneficial microbial diversity and expansion of opportunistic pathogens provide important insights into the pathogenesis of graft-versus-host disease and post-transplant infections. These findings support the potential of microbiome profiling as a predictive biomarker and open new avenues for microbiota-targeted therapeutic strategies to improve transplant success and patient survival.

Introduction

The human gut microbiota is a complex and dynamic community of trillions of microorganisms, including bacteria, archaea, viruses, and fungi, that inhabit the gastrointestinal tract. This microbial ecosystem plays a fundamental role in maintaining host health by contributing to digestion, nutrient absorption, immune system development, and protection against pathogenic organisms. In addition, the gut microbiota is involved in the synthesis of essential vitamins and short-chain fatty acids (SCFAs), as well as in maintaining metabolic homeostasis and mucosal integrity. Through continuous interaction with the host immune system, the gut microbiome influences both innate and adaptive immune responses, thereby playing a critical role in immune tolerance and inflammation control.(1-3)

In the context of allogeneic bone marrow transplantation (allo-BMT), the gut microbiota undergoes profound alterations. Conditioning regimens such as high-dose chemotherapy and total body irradiation, along with the use of broad-spectrum antibiotics and immunosuppressive therapies, significantly disrupt the microbial ecosystem. Additional factors, including dietary modifications and prolonged hospitalization, further contribute to this imbalance. These combined effects lead to microbial dysbiosis, which is characterised by reduced microbial diversity, depletion of beneficial commensal organisms such as *Lactobacillus*, *Bifidobacterium*, and *Clostridia*, and overgrowth of opportunistic pathogens including *Enterococcus*, *Escherichia coli*, and *Clostridioides difficile*.(4-8)

Microbial dysbiosis has been strongly associated with adverse clinical outcomes in BMT patients. The loss of microbial diversity compromises colonisation resistance and intestinal barrier function, thereby increasing susceptibility to infections. Moreover, gut-derived microbial signals can promote inflammatory responses, contributing to the development and severity of acute graft-versus-host disease (GVHD). Dysbiosis is also linked to delayed immune reconstitution and reduced overall survival. Recent advances in high-throughput sequencing and metagenomic analysis have identified specific microbial signatures associated with transplant-related complications. For instance, the presence of *Blautia* has been correlated with reduced GVHD-related mortality, whereas the loss of *Clostridiales* and the overgrowth of *Enterococcus* species have been linked to poorer clinical outcomes, including bloodstream infections.(5,9)

The gut microbiota is closely integrated with host immune regulation, and this relationship becomes particularly critical in the BMT setting. Disruption of the intestinal epithelium due to conditioning regimens, combined with antibiotic-induced depletion of commensal microbes, results in immune dysregulation and a pro-inflammatory environment. This imbalance can exacerbate gastrointestinal GVHD and increase the risk of systemic infections. BMT recipients are particularly vulnerable during the neutropenic phase, during which microbial translocation across the compromised gut barrier may lead to bloodstream infections and colonisation by multidrug-resistant organisms.

Several clinical studies have demonstrated that reduced gut microbial diversity during or after allo-BMT is a strong predictor of poor clinical outcomes, including increased incidence of GVHD, higher infection rates, delayed immune recovery, and decreased survival. For example, patients with lower microbial diversity at the time of neutrophil engraftment have been shown to have a higher risk of transplant-related mortality, whereas greater microbial diversity has been associated with improved overall survival in large multicentre studies. In addition, the use of broad-spectrum antibiotics, particularly those targeting anaerobic commensals, has been linked to increased GVHD-related mortality, highlighting the importance of microbiota-preserving therapeutic strategies.(11-14)

These findings have led to growing interest in microbiota-targeted interventions aimed at restoring microbial balance and improving transplant outcomes. Approaches such as probiotics, prebiotics, faecal microbiota transplantation (FMT), microbiota-sparing antibiotics, and dietary modulation are being explored as potential strategies to maintain or restore gut microbial diversity.

Given the critical role of the gut microbiota in modulating immune responses and clinical outcomes in BMT, a comprehensive understanding of microbiome alterations following transplantation is essential. Therefore, this study aims to investigate the changes in gut microbial composition and metagenomic profiles in allogeneic BMT patients and to explore their potential implications in transplant-related complications.(15)

Materials and Methods

Study Cohort and Sample Collection

The study cohort was designed with reference to previous work investigating gut microbiota alterations in haematopoietic stem cell transplant recipients. A patients undergoing allogeneic bone marrow transplantation (allo-BMT) were included, and fecal samples were collected at defined time points before transplantation (pre-BMT) and following transplantation (post-BMT) to assess microbiome changes.(12)

Ethical approval for the study was obtained from the Institutional Ethics Committee of Genexplore Diagnostics and Research Centre Pvt. Ltd., Ahmedabad. Fecal samples and associated clinical data were collected from patients at the Blood Cancer Institute, Surat. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Fecal specimens were collected within 24 hours of passage and immediately stored at −80 °C until further processing. Samples were collected approximately 30 days post-transplantation, a time point selected to capture early microbiome alterations following conditioning regimens, antibiotic exposure, and immune suppression.

DNA Extraction, PCR Amplification, and Purification

Total genomic DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The quality and quantity of extracted DNA were assessed using a Q5000 micro-volume UV–Vis spectrophotometer (Quawell Technology, San Jose, CA, USA). Extracted DNA samples were stored at −20 °C until further analysis.

Amplification of the bacterial 16S rRNA gene was performed using the Ion 16S Metagenomics Kit, with previously extracted DNA serving as the template. The V2–V3 hypervariable region was amplified using the primer pair U341F-MiSeq and 805R-MiSeq. PCR reactions were carried out in a final reaction mixture containing template DNA and sterile deionised water.

Thermal cycling conditions included an initial denaturation step at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C

for 1 minute, with a final extension at 72 °C for 5 minutes. Negative controls were included in parallel to monitor contamination.

PCR products were resolved on a 1.5% (w/v) agarose gel prepared in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). Amplicons of approximately 550 bp were visualised using a Gel Doc XR+ system (Bio-Rad, USA). Amplified products were purified using a polyethylene glycol (PEG) precipitation method (20% PEG, 2.5 M NaCl), followed by centrifugation at 14,000 × g. The resulting pellets were washed twice with 70% ethanol, air-dried, and resuspended in sterile deionised water. DNA concentration was re-evaluated using the Q5000 spectrophotometer.

Indexing PCR and Library Preparation

Purified PCR amplicons were diluted to a concentration of 10 ng/µL and used as templates for a second round of PCR to incorporate sample-specific index sequences and sequencing adaptors. Indexing PCR was performed using a 16S Metagenomic Sequencing Library Preparation Kit in a final reaction volume of 50 µL.

Each reaction mixture contained 10 µL of 5× buffer, 1.5 µL of dNTPs (10 mM each), 5 µL each of forward and reverse indexing primers (10 µM), 1 µL of DNA polymerase (1 U/µL), 2 µL of template DNA, and nuclease-free water to volume. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 minutes, followed by 8 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes.

Indexed amplicons were purified using the QIAquick PCR Purification Kit and NGS Clean-up and Size Selection Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. Purified libraries were quantified using a Qubit 2.0 fluorometer and pooled in equimolar concentrations (8 nM). Sequencing was performed using a 2 × 300 bp paired-end protocol on an Illumina sequencing platform.

Results

Taxonomic Composition of Gut Microbiota

Comparative analysis of pre- and post-bone marrow transplantation (BMT) samples revealed a marked shift in gut microbial composition. At the phylum level, post-transplant samples were overwhelmingly dominated by Proteobacteria, which increased from 35.82% in pre-transplant samples to 99.33% post-transplant. In contrast, Firmicutes showed a substantial decline from 49.25% to 0.67%, while Bacteroidetes were completely depleted (8.21% to 0.00%).

At lower taxonomic levels, a similar trend was observed. Gammaproteobacteria increased from 31.34% to 99.28%, with Enterobacterial and Enterobacteriaceae also showing a dramatic rise (24.63% to 99.28%), indicating a strong post-transplant enrichment of Proteobacteria-associated taxa.

Category/Species	Pre-sample percentage	Post Sample Percentage
Taxonomic Summary		
Proteobacteria	35.82	99.33
Firmicutes	49.25	0.67
Gammaproteobacteria	31.34	99.28
Enterobacteriales	24.63	99.28
Enterobacteriaceae	24.63	99.28
Bacteroidetes	8.21	0.00
Key Species Changes		

Morganella morganii	1.82	0.02
Prevotella copri	1.79	0.00
Streptococcus salivarius	1.61	0.00
Escherichia coli	0.68	0.09
Serratia rubidaea	0.10	1.36
Klebsiella pneumoniae	0.04	10.33
Klebsiella variicola	0.04	4.83
Lactobacillus salivarius	0.12	0.01
Enterobacter sacchari	0.00	0.99

Table 1. Alterations in gut microbiota composition at taxonomic and species levels following allogeneic BMT

4.2 Species-Level Changes in Microbial Community

Significant alterations were observed at the species level between pre- and post-transplant samples. Pre-transplant samples exhibited a relatively diverse microbial profile, with notable representation of *Morganella morganii* (1.82%), *Prevotella copri* (1.79%), and *Streptococcus salivarius* (1.61%).

Post-transplant samples, however, showed dominance of a limited number of opportunistic and potentially pathogenic species. *Klebsiella pneumoniae* increased markedly from 0.04% to 10.33%, while *Klebsiella variicola* rose from 0.04% to 4.83%. Similarly, *Serratia rubidaea* increased from 0.10% to 1.36%, and *Enterobacter sacchari* emerged in post-transplant samples (0.99%), having been absent in pre-transplant samples.

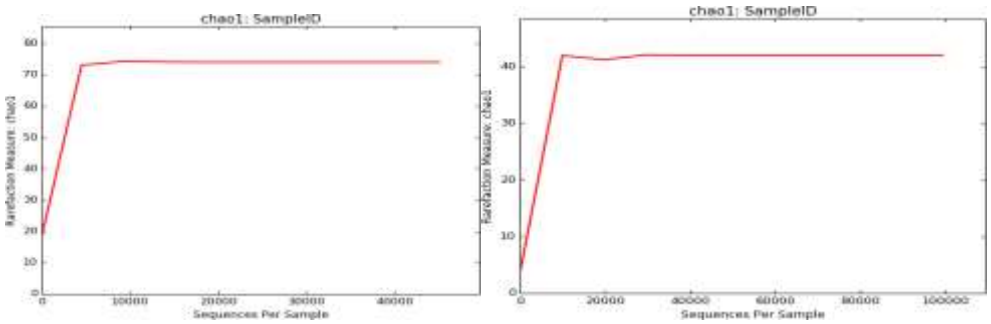
Conversely, several commensal species, including *Prevotella copri*, *Streptococcus salivarius*, and *Lactobacillus salivarius*, were either completely depleted or reduced to negligible levels following transplantation.

Species	Pre Sample Percentage	Post Sample Percentage	Change
Klebsiella pneumoniae	0.04%	10.33%	Massive Increase
Klebsiella variicola	0.04%	4.83%	Massive Increase
Serratia rubidaea	0.10%	1.36%	Significant Increase
Proteobacteria (Over all)	<4% (estimated)	>99% (estimated)	Overwhelming Increase

Table 2. Species-level shifts in gut microbiota composition between pre- and post-BMT samples

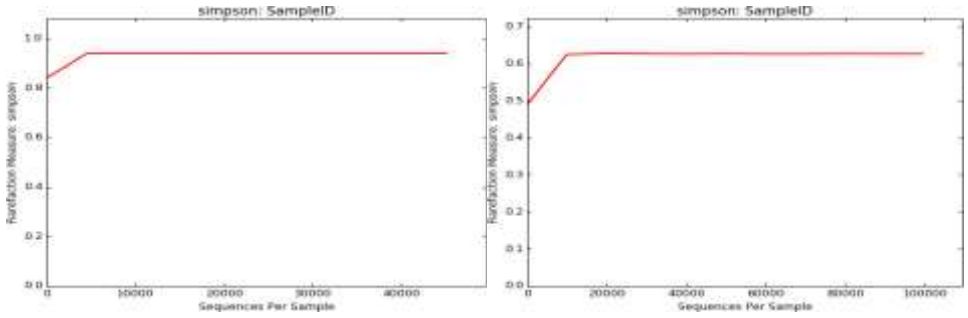
Microbial Diversity Analysis

Alpha diversity analysis demonstrated a substantial reduction in microbial diversity following BMT. Pre-transplant samples exhibited higher species richness and evenness, as indicated by diversity indices such as Chao1, Shannon, and Simpson. In contrast, post-transplant samples showed significantly reduced diversity, reflecting a loss of microbial richness and dominance of a limited number of taxa.



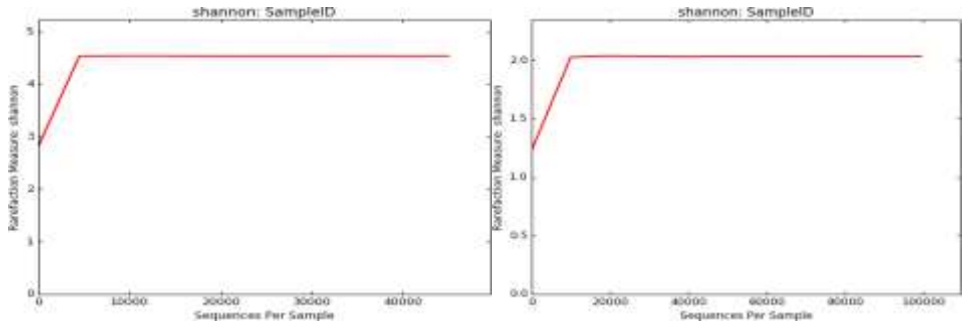
(A) **(B)**

Figure 1 (A) Rarefaction Curves of Pre Bone marrow transplantation patient sample in Chao 1 Microbial diversity. (B) Rarefaction Curves of Post Bone marrow transplantation patient sample in Chao 1 Microbial diversity.



(A) **(B)**

Figure 2 (A) Rarefaction Curves of Pre Bone marrow transplantation patient sample in Simpson Microbial diversity. (B) Rarefaction Curves of Post Bone marrow transplantation patient sample in Simpson Microbial diversity.



(A) **(B)**

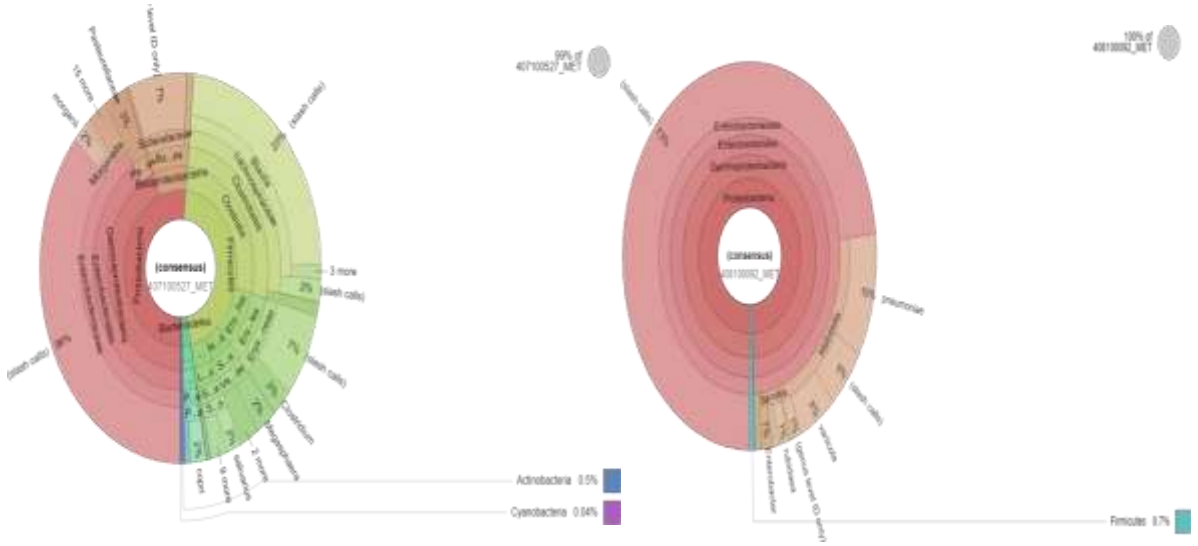
Figure 3 (A) Rarefaction Curves of Pre Bone marrow transplantation patient sample in Shannon Microbial diversity. (B) Rarefaction Curves of Post Bone marrow transplantation patient sample in Shannon Microbial diversity.

Microbial Community Structure (Krona Analysis)

Krona chart visualisation further supported the observed taxonomic shifts. Pre-transplant samples displayed a relatively balanced microbial community, with Proteobacteria (51%) and Firmicutes (46%) as dominant phyla, along with minor contributions from Bacteroidetes (2%).

In contrast, post-transplant samples exhibited near-complete dominance of Proteobacteria (~99%), with minimal representation of other phyla such as Firmicutes (~0.7%). This indicates a transition from a

diverse microbial ecosystem to a highly simplified and imbalanced community structure.



(A) **(B)**

Figure 4 (A) Krona chart of the full taxonomy of Pre Bone marrow transplantation patient sample. (B) Krona chart of the full taxonomy of Post Bone marrow transplantation patient sample.

Overall Microbial Shift Following BMT

Overall, the results demonstrate a significant shift from a diverse and balanced gut microbiota in pre-transplant samples to a highly dysbiotic state post-transplant. This shift is characterised by:

- Loss of beneficial commensal bacteria
- Expansion of opportunistic and potentially pathogenic species
- Marked reduction in microbial diversity
- Dominance of Proteobacteria

Discussion

The present study demonstrates substantial disruption of the gut microbiota following allogeneic bone marrow transplantation, indicating a transition from a diverse microbial ecosystem to a dysbiosis state. This alteration is characterised by a predominance of Proteobacteria and depletion of key commensal taxa, reflecting a major imbalance in microbial homeostasis.

The expansion of Proteobacteria observed in this study is consistent with previously reported findings and is widely recognised as a marker of intestinal dysbiosis and inflammation. Members of this phylum are often associated with opportunistic infections and are known to thrive under conditions of antibiotic exposure and immune suppression. Their dominance may contribute to impaired gut barrier function and increased susceptibility to systemic infections.(4,9)

In contrast, the reduction of Firmicutes and complete depletion of Bacteroidetes indicate a loss of beneficial microbial populations that play a crucial role in maintaining gut health. These taxa are important producers of short-chain fatty acids, which are essential for epithelial integrity and immune regulation. Their depletion may therefore promote a pro-inflammatory environment and increase the risk of complications such as graft-versus-host disease.

At the species level, the enrichment of opportunistic pathogens, including *Klebsiella pneumoniae*,

Klebsiella variicola, and *Serratia rubidaea*, suggests a shift towards a pathogenic microbiome following transplantation. These organisms are frequently associated with hospital-acquired infections and antimicrobial resistance, highlighting their clinical relevance in immunocompromised patients. The emergence of additional taxa exclusively in post-transplant samples further supports the role of transplantation-related factors in shaping microbial composition.

Conversely, the observed decline in commensal species such as *Prevotella copri*, *Streptococcus salivarius*, and *Lactobacillus salivarius* suggests a loss of protective microbial functions. These organisms contribute to microbial balance, metabolic activity, and immune modulation, and their absence may facilitate pathogen overgrowth and immune dysregulation.

The reduction in microbial diversity further supports the presence of dysbiosis. Lower diversity has been consistently associated with adverse clinical outcomes in transplant recipients, including increased incidence of graft-versus-host disease, delayed immune recovery, and reduced survival. These findings are in agreement with previous studies demonstrating that microbial diversity is a key determinant of transplant success. (11-14)

The underlying mechanisms driving these changes are likely multifactorial. Conditioning regimens, antibiotic exposure, and immunosuppressive therapy disrupt the intestinal environment, leading to loss of commensal bacteria and expansion of resistant organisms. Damage to the intestinal epithelium may also facilitate microbial translocation and systemic inflammation, further exacerbating complications.

From a clinical perspective, these findings highlight the potential utility of gut microbiota profiling as a biomarker for early detection of transplant-related complications. In addition, strategies aimed at preserving or restoring microbial diversity, including microbiota-targeted therapies, may offer promising approaches to improve patient outcomes. (15)

However, this study has certain limitations. The relatively small sample size may limit the generalisability of the findings, and the analysis was restricted to a single post-transplant time point. Future studies incorporating larger cohorts and longitudinal sampling are required to better understand the dynamics of microbiome alterations and their direct clinical implications.

In summary, this study provides evidence of significant gut microbiota dysbiosis following allogeneic bone marrow transplantation, characterised by reduced diversity, loss of beneficial microbes, and expansion of opportunistic pathogens. These alterations may contribute to transplant-related complications and underscore the importance of microbiome-focused approaches in the management of BMT patients.

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Data Availability

The datasets generated and/or analyzed during the current study are not publicly available at this stage as they form part of an ongoing PhD project. Data will be made publicly available upon completion of the

study and will be deposited in the NCBI Sequence Read Archive repository.
In the interim, data are available from the corresponding author on reasonable request, subject to institutional and ethical approvals.

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