REVIEW

Enteric dysbiosis in liver and kidney transplant recipients: a systematic review

Saranya Sivaraj[1], Anita Chan[1], Elisa Pasini[1], Emily Chen[1], Bishoy Lawendy[1], Elizabeth Verna[2], Kymberly Watt[3] & Mamatha Bhat[1,4]

1 Multi Organ Transplant Program, University Health Network, Toronto, ON, Canada
2 Division of Digestive and Liver Diseases, Center for Liver Disease and Transplantation, Columbia University Irving Medical Center, New York, NY, USA
3 Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA
4 Division of Gastroenterology and Hepatology, University Health Network and University of Toronto, Toronto, ON, Canada

Correspondence
Dr. Mamatha Bhat MD, MSc, PhD, FRCPC, Staff Hepatologist and Clinician-Scientist, Multiorgan Transplant Program, University Health Network, Assistant Professor, Division of Gastroenterology, University of Toronto, 585 University Avenue, 11PMB-318, Toronto, Ontario M5G 2N2, Canada
Tel.: 416-340-4800;
fax: 416-340-4043;
e-mail: mamatha.bhat@uhn.ca

*Equal contribution.

SUMMARY

Several factors mediate intestinal microbiome (IM) alterations in transplant recipients, including immunosuppressive (IS) and antimicrobial drugs. Studies on the structure and function of the IM in the post-transplant scenario and its role in the development of metabolic abnormalities, infection, and cancer are limited. We conducted a systematic review to study the taxonomic changes in liver (LT) and kidney (KT) transplantation, and their potential contribution to post-transplant complications. The review also includes pre-transplant taxa, which may play a critical role in microbial alterations post-transplant. Two reviewers independently screened articles, and assessed risk of bias. The review identified 13 clinical studies, which focused on adult kidney and liver transplant recipients. Patient characteristics and methodologies varied widely between studies. Ten studies reported increased an abundance of opportunistic pathogens (*Enterobacteriaceae*, *Enterococcaceae*, *Fusobacteriaceae*, and *Streptococcaceae*) followed by butyrate-producing bacteria (*Lachnospiraceae* and *Ruminococcaceae*) in nine studies in post-transplant conditions. The current evidence is mostly based on observational data and studies with no proof of causality. Therefore, further studies exploring the bacterial gene functions rather than taxonomic changes alone are in demand to better understand the potential contribution of the IM in post-transplant complications.

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Key words
immunosuppression, intestinal microbiome, kidney transplantation, liver transplantation, opportunistic pathogens, post-transplant complications

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Introduction

Solid organ transplantation (SOT) represents a life-saving intervention for those with end-stage organ disease. The use of maintenance immunosuppression and perioperative antibiotic prophylaxis is crucial for graft health and patient longevity. However, continued use of immunosuppression is associated with metabolic syndrome (MS), infections, de novo/recurrent fatty liver disease, and malignancies [1,2]. The MS, which includes obesity, hyperglycemia, dyslipidemia, and hypertension together with immunosuppressants, plays an important role in development of cardiovascular disease in transplant recipients [3,4]. Given that, immunosuppressants and perioperative antibiotics along with transplantation procedures have been reported to induce intestinal microbiome (IM) alterations [5]. In the non-transplant population,

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microbiome alterations are associated with several conditions, including metabolic disorders, autoimmune diseases, inflammatory bowel disease, neurological conditions, and cancer [6–9].

The IM is comprised of the genomes 10^{14} microorganisms, including bacteria, fungi, protozoans, and viruses that live symbiotically in the human gastrointestinal tract [10]. Bioactive metabolites produced from microbial metabolism influence host metabolism and immunity by activating cellular pathways and targets [11]. As a result, the microbiome is involved in many vital processes including digestion, sucrose degradation, *de novo* synthesis of essential vitamins, and detoxification [12]. Maintaining the relative abundance of each component within the microbiome is critical to health. Dysbiosis is defined as the alteration of intestinal microbiome and loss of diversity associated with post-transplant infections [40]. Dysbiosis disrupts the integral networks within the host and consequently results in number of diseases, many of which affect patients after solid organ transplantation [10,13].

A number of human and animal studies have investigated the microbial alterations in the presence of IS. Tourret *et al*. [14] demonstrated the overgrowth of *Escherichia coli* and increased colonization of opportunistic pathogens in mice exposed to everolimus, mycophenolate mofetil (MMF), tacrolimus, and prednisolone. In addition, mice treated with MMF were associated with shift in the microbial composition and colonization of pathogenic bacteria such as *Escherichia/Shigella* together with enrichment of lipopolysaccharide biosynthesis and β-glucuronidase, resulting in inflammation [15,16]. Two other studies on murine models reported alterations in the relative abundance of taxa within the microbiome and induced insulin resistance after the initiation of IS agents [17,18]. More specifically, our group has demonstrated the effect of immunosuppression on the metagenomic composition of the IM in rats that is reversed with the use of probiotics, suggesting a contributory role for the microbiome in PTDM [17].

We performed a systematic review to study the alteration of intestinal microbial composition in Liver and kidney transplant recipients (before and after transplantation), and why these may contribute to post-transplant complications. We decided to focus on liver and kidney transplant recipients, given the significant amount of literature that has accumulated in these two transplant patient populations.

Methods

Literature search

We used the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) guidelines to perform this systematic review [19]. Two independent literature searches for papers up to December 31st, 2019, were conducted on PubMed. Search MeSH terms including "intestinal microbiome", "transplant", "immunosuppression", and individual IS drugs (i.e., tacrolimus, cyclosporine, prednisone, sirolimus, everolimus, and mycophenolate mofetil) were used to identify all clinical studies in SOT that investigated the effects of immunosuppression on the microbiome. Details of the search and MeSH terms are listed in Table S1. Eligibility criteria are as follows: (i) articles published in English only; (ii) clinical studies; and (iii) IM from stool samples and rectal swabs (Fig. 1). The summary of the included studies and its outcomes can be found in Table S2.

Exclusion criteria

Clinical studies that utilized immunosuppressive therapy in the management of other solid organ transplant recipients (lung, heart, small bowel, pancreas), inflammatory bowel disease, hematopoietic stem cell transplant, fecal microbiota transplant, and graft-versus-host disease were excluded. Pediatric studies were also excluded.

Data extraction and analyses

Abstracts deemed to be relevant were then subjected to a full-text review. For each included article, details regarding study design, population characteristics, diversity, and microbiome composition pre- and post-transplant were extracted and summarized. The Newcastle–Ottawa Scale (NOS) for evaluating the quality of non-randomized studies in meta-analyses was used to assess the quality of the studies [20]. NOS contains three sections to score the quality of the included studies: (i) selection, (ii) comparability, and (iii) outcome. We rated the quality of the studies (good, fair, and poor) based on the total obtained from the three sections. A "good" quality score requires more than or equal to total of 7. A "fair" quality score required more than or equal to total of 5. A "poor" quality score reflected less than or equal to 4 (Table 1). The microbiome composition from each study was then classified according to the taxonomic hierarchy. As per previously published

Transplant International 2020; 33: 1163–1176

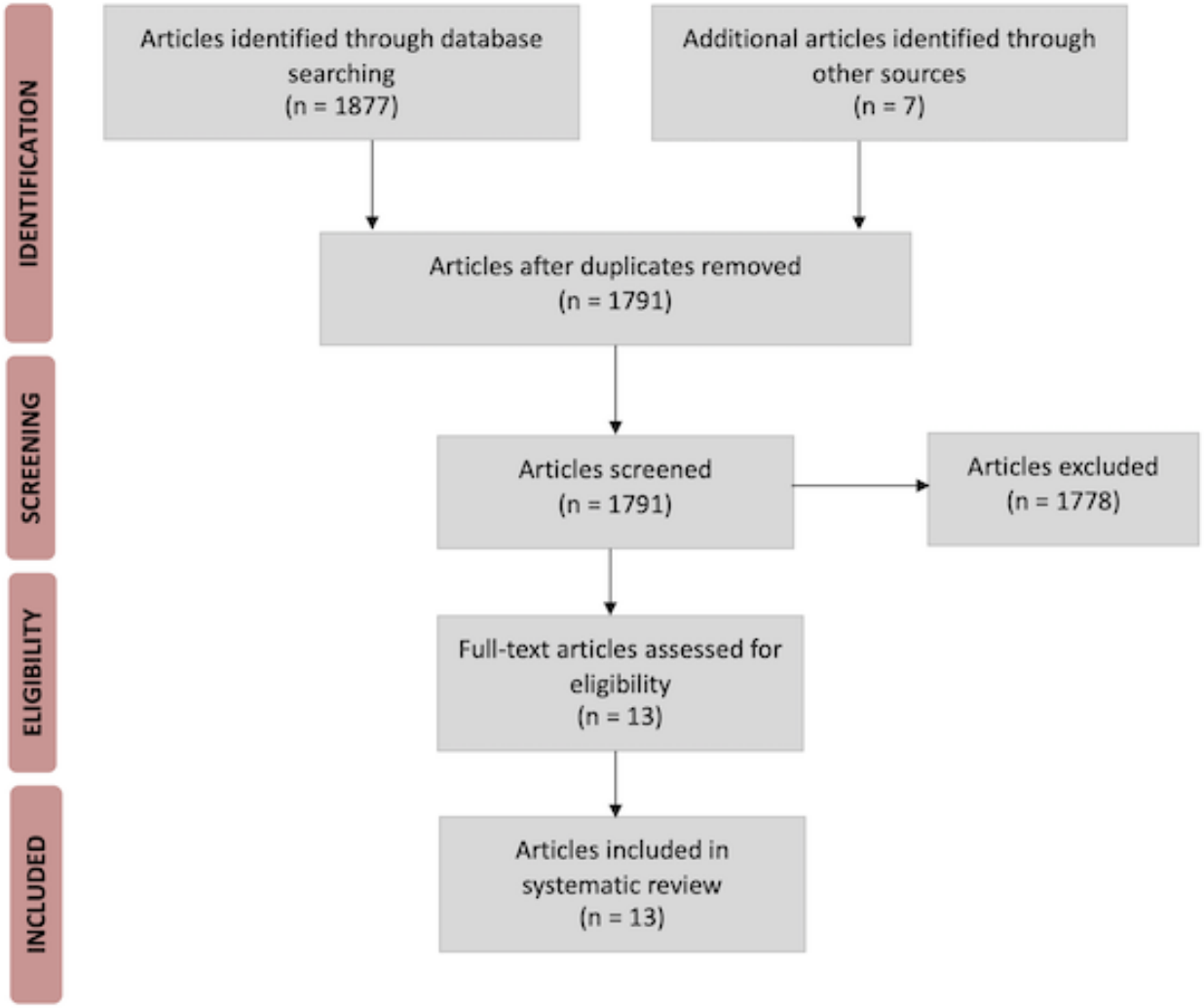

Figure 1 Flowchart of literature search for articles including clinical studies of intestinal microbiome in solid organ transplant recipients in comparison with healthy population data or pre-transplant data.

methodology [21], the microbial presence of each microorganism was determined by counting its frequency, and the corresponding order or family was identified in the pre- or post-transplant microbiome in each of the 13 included studies. Microbial predominance was determined by identifying the most abundant order or family among each of the included studies. The comparison of the microbial families identified in the pre- and post-transplant populations from both kidney and liver studies was performed using a Venn diagram (Fig. 2) [22].

Results

A total of 1877 abstracts were identified, and 93 duplicates were removed. After the application of exclusion criteria and review of full-text articles, six clinical studies were eligible for inclusion. From hand-searching, seven studies were identified for further screening (Fig. 1). We identified ten liver and three kidney transplant studies. The characteristics of the included human studies, methodology, and the taxa of each study can be found in Tables 2 and 3. The indication for IS therapy in all of the included studies was to prevent graft rejection following SOT. The maintenance IS agents used following transplantation included tacrolimus, cyclosporine, sirolimus, everolimus, mycophenolate mofetil, and prednisone.

Overall, five studies collected pre- and post-transplant fecal samples, while eight studies collected only post-transplant samples. The range of time from transplant to fecal sample collection was 3 weeks to 4.7 years. Eight studies had 16S rRNA sequencing of genetic material extracted from fecal samples using Illumina Hi/

Table 1. Risk of bias assessment of the included studies using the New Castle–Ottawa (NOS) scale.

First author, year of publication	Type of study	Selection	Comparability	Outcome	Total score	Quality
Liver transplantation studies						
Annvajhala, 2019 [23]	Cohort	4	2	3	9	Good
Bajaj, 2018 [24]	Cohort	4	2	2	8	Good
Kato, 2017 [25]	Cohort	4	2	3	9	Good
Sun, 2017 [26]	Case–Control	4	2	2	8	Good
Lu, 2019 [27]	Case–Control	4	2	2	8	Good
Kabar, 2015 [35]	Cohort	2	0	2	4	Poor
Lu, 2013 [28]	Case–Control	4	1	2	7	Good
Macesic, 2018 [32]	Cohort	3	0	3	6	Fair
Wu, 2012 [29]	Case–Control	4	1	2	7	Good
Zhang, 2017 [30]	Case–Control	4	2	2	8	Good
Kidney transplantation studies						
Fricke, 2014 [31]	Cohort	4	2	3	9	Good
Lee, 2015 [33]	Cohort	3	0	2	5	Fair
Zaza, 2017 [34]	Cohort	3	0	2	5	Fair

Quality ≥7: Good; Quality ≥5: Fair; Quality ≤4: Poor.

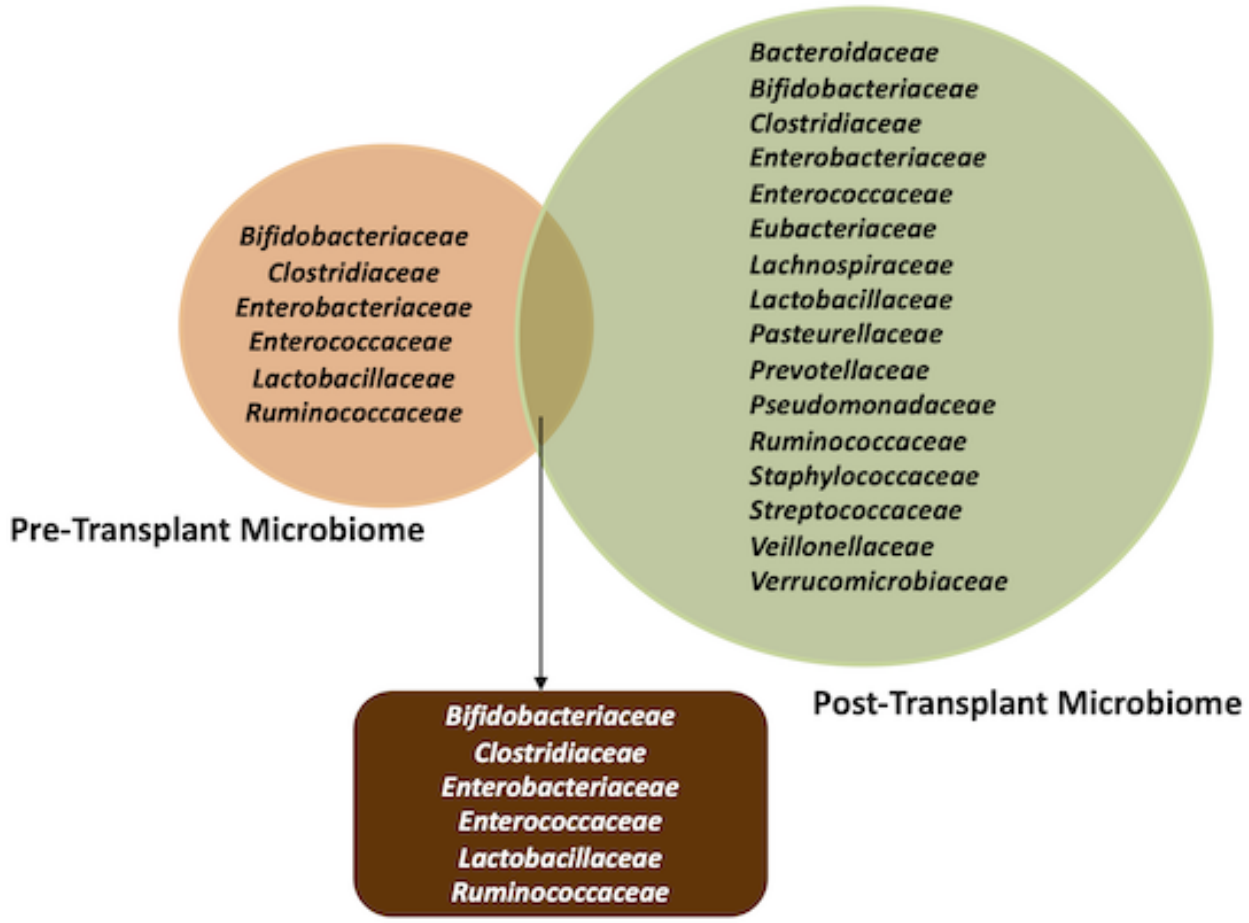

Figure 2 Comparison of pre- and post-transplant bacterial families identified to be predominant in more than one liver or kidney study.

Transplant International 2020; 33: 1163–1176

Table 2. Methodologic characteristics and taxa representation in 10 liver transplant studies.

First author, year of publication	Population characteristics	Sample	Antimicrobial drugs	Immunosuppressants	Sequencing platform	Hypervariable region	Pre-LT (family)	Post-LT (family)
Analysis of pre- and post-transplant microbiome								
Annavajhala, 2019 [23]	195 adults (107 male); median age 60, 177 patients; Indication for transplant: hepatitis C (n = 71), NAFLD (n = 30), alcohol-related liver disease (n = 19), HCC (n = 69)	Stool	Aminoglycoside Cephalosporins Carbapenem Daptomycin Fluoroquinolone Piperacillin–tazobactam Vancomycin	Not listed in the study	Illumina MiSeq or HiSeq	V3/V4	*Bifidobacteriaceae* ↑ *Enterococcaceae* ↑ *Lactobacillaceae* ↑ *Ruminococcaceae* ↑ *(low MELD)* *Ruminococcaceae* ↓ *(ARLD)* *Streptococcaceae* ↑ *Veillonellaceae* ↑	*Bacteroidaceae* ↓ *Clostridiaceae* ↑ *Enterobacteriaceae*↑ *Enterococcaceae* ↓ *Lachnospiraceae* ↑ *Lactobacillaceae* ↓ *Ruminococcaceae* ↑ *Streptococcaceae* ↓
Bajaj, 2018 [24]	40 adults (35 males); mean age 56 ± 7; Indication for transplant: hepatitis C (n = 17), hepatitis C and alcohol (n = 8), NASH (n = 6), and others (n = 9)	Stool	Trimethoprim Sulfamethoxazole	Tacrolimus Cyclosporine MMF	Multi-Tag Sequencing	NA	*Bifidobacteriaceae* ↑ *Enterobacteriaceae* ↑	*Clostridiaceae* ↑ *Sutterellaceae* ↑ *Enterobacteriaceae* ↓ *Lachnospiraceae* ↑ *Ruminococcaceae* ↑
Kato, 2017 [25]	38 adults (24 males); mean age 50.4 ± 2.4; Indication for transplant: Leading cause of cirrhosis was Hepatitis B or C virus (n = 14), post-kasai biliary atresia (n = 6), alcoholic cirrhosis (n = 6), primary biliary cirrhosis (n = 4), NASH (n = 4) and other (n = 4)	Stool	Cefotaxime Ampicillin Rituximab	Tacrolimus MMF	Illumina MiSeq	V3–V4	Low diversity: in high meld score patients compared to low meld score High meld and CPS: *Enterobacteriaceae* ↑ *Enterococcaceae* ↑ *Lactobacillaceae* ↑ *Bacteroidaceae* ↓ *Lachnospiraceae* ↓ *Ruminococcaceae* ↓	*Bacteroidaceae* ↑ *Bifidobacteriaceae* ↑ *Clostridiaceae* ↑ *Enterobacteriaceae* ↑ *Enterococcaceae* ↑ *Lactobacillaceae* ↑ *Peptostreptococcaceae* ↑ *Ruminococcaceae* ↑ *Streptococcaceae* ↑

Table 2. Continued.

First author, year of publication	Population characteristics	Sample	Antimicrobial drugs	Immunosuppressants	Sequencing platform	Hypervariable region	Pre-LT (family)	Post-LT (family)
Sun, 2017 [26]	9 adults (9 males); mean age 49.4; Indication for transplant: Decompensated Cirrhosis (n = 4), and HCC (n = 5). HC = 15	Stool	No antibiotic therapy 1 month prior to LT 5–7 days after surgery Cephalosporin	Tacrolimus MMF	Illumina MiSeq	V4	*Aeromonadaceae* ↑ *Anaerolineaceae* ↑ *Clostridiaceae* ↑ *Enterobacteriaceae* ↑ *Fusobacteriaceae* ↑ *Pasteurellaceae* ↑	*Aeromonadaceae* ↓ *Anaerolineaceae* ↓ *Chitinophagaceae* ↑ *Clostridiaceae* ↓ *Coriobacteriaceae* ↑ *Desulfobacteriales* ↑ *Enterobacteriaceae* ↓ *Eubacteriaceae* ↑ *Fusobacteriaceae* ↓ *Micromonosporaceae* ↑ *Pasteurellaceae* ↓ *Verrucomicrobiaceae* ↑ *Lachnospiraceae*↑* *Corynebacteriaceae*↑*
Analysis of post-transplant microbiome								
Lu, 2019 [27]	151 adults (LT patients = 90, HC = 61); age 35–65 years; Indication for transplant = HBV associated HCC	Stool Post-LT >24 months and <48 months	No antibiotics prior to 12 weeks	Tacrolimus	Illumina MiSeq	V3–V4	NA	*Bacteroidaceae* ↑* *Fusobacteriaceae* ↑* *Lachnospiraceae* ↑* *Ruminococcaceae*↑* *Lactobacillaceae* ↑* *Streptococcaceae* ↑* *Veillonellaceae* ↑* *Enterococcaceae*↑* *Erysipelotrichaceae*↑* *Enterobacteriaceae* ↑*
Kabar, 2015 [35]	38 adults (25 males); mean age 52.2; Indication for transplant: alcohol (n = 15), HCC (n = 8), Hepatitis B/C (n = 5), Cholestatic liver disease (n = 5), other (n = 5)	Bile & Stool	Ciprofloxacin Imipenem Piperacillin–tazobactam	Tacrolimus Cyclosporine Everolimus Sirolimus MMF	Agar Method	NA	NA	*Enterobacteriaceae* *Enterococcaceae* *Pseudomonadaceae* *Staphylococcaceae*

Table 2. Continued.

First author, year of publication	Population characteristics	Sample	Antimicrobial drugs	Immunosuppressants	Sequencing platform	Hypervariable region	Pre-LT (family)	Post-LT (family)
Lu, 2013 [28]	12 Adults (12 males); mean age 39; Indication for transplant: HBV (n = 12), asymptomatic adult carries of HBV with mean age 35.2 years as controls (n = 5)	Stool	Piperacillin–tazobactam, Cefepime dihydrochoride Imipenem-cilastatin sodium Cefoperazone sodium Sulbactam sodium Micafungin sodium Caspofungin acetate Vancomycin Teicoplanin Flucytosine amphotericin B	Tacrolimus Simulect MMF Glucocorticoids	Denaturing Gradient Gel Electrophoresis	V3	NA	*Bacteroidaceae* *Clostridiaceae* *Enterobacteriaceae* *Enterococcaceae* *Fusobacteriaceae* *Lactobacillaceae* *Porphyromonadaceae* *Prevotellaceae* *Pseudomonadaceae* *Streptococcaceae* *Veillonellaceae*
Macesic, 2018 [32]	142 adults; median age 60.4; 128 adults included in the analysis: hepatitis C (n = 49), NAFLD (n = 18), Autoimmune hepatitis (n = 16), Alcoholic liver disease (n = 14), Hepatitis B (n = 9), Fulminant hepatic failure (n = 2)	Stool and/or rectal swabs	NA	Not listed in the study	Illumina Miseq and HiSeq	Whole genome sequencing	NA	*Enterobacteriaceae*
Wu, 2012 [29]	190 adults (169 male); mean age 41.9, Liver Transplant (n = 111) Liver cirrhosis (n = 51), Healthy control (n = 28) Indications for transplant: decompensated HBV Acute or chronic HBV liver failure	Stool	No antibiotics	Glucocorticoid MMF Cyclosporine Tacrolimus	qPCR	NA	NA	*Eubacteriaceae*↓* *Bifidobacteriaceae*↓* *Lachnospiraceae*↓* *Lactobacillaceae*↓* *Enterobacteriaceae* ↑* *Enterococcaceae* ↑* *Prevotellaceae* ↑*

Table 2. Continued.

First author, year of publication	Population characteristics	Sample	Antimicrobial drugs	Immunosuppressants	Sequencing platform	Hypervariable region	Pre-LT (family)	Post-LT (family)
Zhang, 2017 [30]	30 adults (25 males); mean age 41; indication for transplant: NAS (n = 10), patients with no complication post-LT (n = 10), non-LT healthy (n = 10)	Stool	No history of antibiotics within previous 3 months	Tacrolimus MMF Methylprednisolone	Illumina MiSeq	V3–V4	NA	*Ruminococcaceae* ↓* *Lachnospiraceae* ↓* *Enterococcaceae* ↑* *Streptococcaceae* ↑* *Pseudomonadaceae*↑* *Verrucomicrobiaceae* ↑* *Prevotellaceae* ↓* *Bacteroidaceae* ↓*

↑*, taxa enriched in comparison with healthy controls; ↑, taxa enriched in comparison with pre-/post-transplant taxa; ↓*, taxa decreased in comparison with healthy controls; ↓, taxa decreased in comparison with pre-/post-transplant taxa; ARLD, alcohol-related liver disease; HBV, hepatitis B viral infection; HC, healthy controls; HCC, hepatocellular carcinoma; LT, liver transplant; MELD, model for end-stage liver disease; MMF, Mycophenolate mofetil; NA, not available; NAFLD, non-alcoholic fatty liver disease; NAS, non-anastomotic stricture; NASH, non-alcoholic steatohepatitis.

MiSeq platform as the method of taxonomic identification. One study with Denaturing Gradient Gel Electrophoresis (DGGE) of the V3 hypervariable region and the remaining four studies had pyrosequencing, agar method, multi-tag sequencing, and qPCR. Table 1 provides quality scores for the studies, assessing risk of bias. In total, nine studies were of good quality [23–31], three studies were of fair quality [32–34], and one was of poor quality [35]. The main concerns were outcome (lack of adequate follow-up) and comparability.

Pre-liver transplant microbiome

The analysis included 4 studies and identified taxa at the family level. *Enterobacteriaceae* was identified in three of the four studies and found to be the most predominant. Other notable families *Bifidobacteriaceae*, *Ruminococcaceae*, *Lactobacillaceae*, and *Enterococcaceae* were identified in 2 studies. *Streptococcaceae*, *Aeromonadaceae*, *Anaerolineaceae*, *Clostridiaceae*, *Fusobacteriaceae*, and *Pasteurellaceae* were identified in one study (Table 2).

Pre-kidney transplant microbiome

One of the three studies included a pre-transplant microbiome. Families such as *Clostridiaceae*, *Erysipelotrichaceae*, *Lachnospiraceae*, and *Peptostreptococcaceae* were less abundant (Table 3).

Post-liver transplant microbiome

Table 2 summarizes the changes that occurred in the bacterial composition of the post-liver transplant microbiome. A total of 26 families and three orders were identified in 10 studies. The family *Enterobacteriaceae* was increased in the post-transplant microbiome in the majority of studies. Other taxa included *Clostridiaceae* and *Enterococcaceae*, which were increased post-LT in 5 studies. *Streptococcaceae* was increased in 4 studies. Additionally, the families *Ruminococcaceae* and *Lachnospiraceae* were found to be increased in four studies (Table 2). *Bacteroidaceae* and *Lactobacillaceae* were increased in three studies. Further, two studies demonstrated increase in the family *Fusobacteriaceae*, *Prevotellaceae*, and *Pseudomonadaceae*.

Post-kidney transplant microbiome

A total of 20 families were identified in three kidney transplant studies. *Bifidobacteriaceae*, *Lachnospiraceae*,

Table 3. Methodologic characteristics and taxa representation in three kidney transplant studies

First author, year of publication	Population characteristics	Sample	Antimicrobial drugs	Immunosuppressants	Sequencing platform	Hypervariable region	Pre-KT [family (genus)]	Post-KT (family)
Analysis of pre- and post-transplant microbiome								
Fricke, 2014 [31]	60 adults (39 males); Mean age 58; indication for transplant = NA	Oral & Rectal Swabs	Cefazolin Penicillin Ciprofloxacin Sulfamethoxazole –trimethoprim	Simulect Thymoglobulin Campath	454 sequencing	V1–V3	*Clostridiaceae* (*Genus: Anaerotruncus*) ↓ *Erysipelotrichaceae* (*Genus: Coprobacillus*) ↓ *Lachnospiraceae* (*Genus: Coprococcus*) ↓ *Peptostreptococcaceae* ↓	*Bacteroidaceae* ↑ *Bifidobacteriaceae* ↑ *Campylobacteraceae* ↑ *Clostridiaceae* ↑ *Corynebacteriaceae* ↑ *Enterobacteriaceae* ↑ *Erysipelotrichaceae* ↑ *Lachnospiraceae* ↑ *Leuconostocaceae* ↑ *Pasteurellaceae* ↑ *Peptostreptococcaceae* ↑ *Porphyromonadaceae* ↑ *Rikenellaceae* ↑ *Ruminococcaceae* ↑ *Staphylococcaceae* ↑ *Veillonellaceae* ↑ *Verrucomicrobiaceae* ↑
Analysis of post-transplant microbiome								
Lee, 2015 [33]	19 adults (8 males); mean age 54.7; indication for transplant = NA	Stool	Amoxicillin Clindamycin Cefazolin Vancomycin	Tacrolimus MMF	Illumina MiSeq	V4–V5	NA	*Bifidobacteriaceae* *Clostridiaceae* *Eubacteriaceae* *Lachnospiraceae* *Ruminococcaceae* *Streptococcaceae*
Zaza, 2017 [34]	20 adults (16 Males); mean age 62.3; Indication for transplant = NA	Stool	NA	Everolimus Tacrolimus MMF Prednisone	Illumina HiSeq	NA	NA	*Bacteroidaceae* *Bifidobacteriaceae* *Coriobacteriaceae* *Enterobacteriaceae* *Eubacteriaceae* *Lachnospiraceae* *Pasteurellaceae* *Ruminococcaceae* *Streptococcaceae*

↑, taxa decrease in comparison with pre-/post-transplant taxa; ↑, taxa enriched in comparison with pre-/post-transplant taxa; KT, kidney transplant; MMF, mycophenolate mofetil; NA, not available.

and *Ruminococcaceae* were found in all three studies. *Bacteroidaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Eubacteriaceae*, and *Streptococcaceae* were identified in two studies (Table 3).

Pre- and post-transplant microbial diversity and predominance

Analysis of microbial diversity and predominance was feasible only for 4 (three liver and one kidney) of the 13 studies, due to lack of data availability. An analysis (Table 4) shows the predominant microorganisms as well as diversity comparisons of the pre- and the post-transplant microbiome from liver and kidney studies. There was an overall decrease in microbial diversity post-transplant as compared to the pre-transplant state [25,28,30,31]. Additionally, an increase in the relative abundance of pathogenic microorganisms, belonging to the phyla *Proteobacteria* and *Actinobacteria*, was noted with antibiotic use despite the decrease in overall diversity. Bajaj *et al*. [24] noted a significant increase in diversity when comparing SDI pre-transplant and at 7 months post-transplant. Kato *et al*. [25] revealed a drop in microbial diversity from a median SDI of 3–4 pre-transplant to 2–3 at 14 days post-transplant. In the kidney study, Fricke *et al*. [31] revealed a significant reduction in the SDI from an interquartile range of 3–5 pre-transplant to 2–5 at 1 month post-transplant. However, the microbial diversity at 15–21 months post-transplant is still lower when compared to healthy controls [30].

The overall presence of bacterial families in the pre- and post-transplant from kidney and liver studies was compared using a Venn diagram, and the results revealed the increased presence of *Bifidobacteriaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, and *Ruminococcaceae* in both before transplant and after transplant (Fig. 2).

Discussion

Our systematic review reveals alterations in microbial composition and an increase in pathogenic taxa in liver and kidney transplant recipients. These data provide a foundation to deepen our knowledge on the impact of SOT-mediated enteric dysbiosis on post-transplant complications.

Many previous studies observed overall loss of microbial diversity has been associated with immune-related diseases, metabolic disease, and cancer in the general population [36–39]. In SOT population, pre-transplant microbiota loss or alteration is influenced by several factors such as malnutrition, infection, primary indication for transplantation, and the transplantation procedure [40]. Other notable factors include immunosuppressants, prophylactic antibiotics, and steroids (Fig. 3). Alterations or loss in microbial diversity increase the risk of post-transplant infection and graft rejection [5,41] particularly KT recipients demonstrated acute rejection, diarrhea, and urinary tract infection [33]. Further, LT recipients reported to have increased endotoxin levels in blood samples compared to healthy individuals and increased intestinal permeability and endotoxemia due to long-term use of tacrolimus [42,43].

Immunosuppression is critical to graft health free of rejection. However, it plays an essential role in the incidence of opportunistic infections [44]. Our analysis revealed the increased presence of *Enterobacteriaceae* post-transplant in the majority of included studies. Though *Enterobacteriaceae* is a part of the commensal IM, its increased presence leads to urinary tract, lower respiratory tract, and bloodstream infections. Lu *et al*. [27] reported that an increase in opportunistic pathogens in transplant recipients was associated with abnormal liver enzymes post-transplant. Particularly, *Enterobacteriaceae* enrichment was associated with endotoxemia, increased intestinal permeability, and liver-related diseases [45,46]. Other pathogenic families such as *Enterococcaceae*, *Streptococcaceae*, and *Pseudomonodaceae* were reported to be associated with non-anastomotic biliary strictures post-LT [30]. More specifically, *Enterococcaceae* was reported to be abundant in children with Crohn's disease treated with Infliximab and insulin-using cirrhotic patients [47,48].

The short-chain fatty acid-producing bacteria *Lachnospiraceae* and *Ruminococcaceae* were found to be increased in the transplant recipients. Interestingly, our previous study on Sprague-Dawley rats exposed to tacrolimus and sirolimus induced hyperglycemia [17]. A linear discriminant analysis (LDA) identified the *Lachnospiraceae* and *Verrucomicrobiaceae* families to be abundant in the immunosuppressed rats compared to control. The abundance of bacteria producing short-chain fatty acids plays an essential role in intestinal inflammation and host resistance [40]. Moreover, *Lachnospiraceae* is widely studied for its role in metabolic disorders and cardiovascular health [49,50]. The increased abundance of pathogenic taxa *Enterobacteriaceae* and *Enterococcaceae* along with the short-chain fatty acid-producing bacteria *Lachnospiraceae* and *Ruminococcaceae* may play a role in post-transplant complications.

Decreased IM diversity post-transplantation has been associated with several complications including

Transplant International 2020; 33: 1163–1176

Table 4. Pre- and post-transplant microbial diversity and predominance from Liver and kidney transplant studies.

First author, year of publication	Microbial diversity		Microbial predominance	
	Pre-transplant	Post-transplant	Pre-transplant (order)	Post-transplant (family)
Liver transplantation studies				
Bajaj, 2018 [24]	SDI [mean (±SD)] 1.6 ± 0.7	SDI [mean (±SD)] 2.1 ± 0.7 Significant increase	*Enterobacteriales*	*Lachnospiraceae*
Kato, 2017 [25]	SDI IQR 0–5 Median SDI: 3–4	SDI IQR 0–4 Median SDI: 2–3 No difference in diversity pre-transplant and at 0–7 days post-transplant. Mean diversity index decreased at days 8–14 post-transplant Significant reduction	*Burkholderiales* *Campylobacterales* *Desulfovibrionales Neisseriales* *Enterobacteriales Bifidobacteriales* *Verrucomicrobiales Synergistales Xanthomonadales* *Pseudomonadales Pasteurellales Aeromonadales* *Clostridiales* *Erysipelotrichales Turicibacterales Lactobacillales* *Gemellales* *Bacillales Bacteroidales Coriobacteriales* *Actinomycetales Methanobacteriales Anaerolineales* *Fusobacteriales* *Rhizobiales* *Sphingomonadales*	*Lachnospiraceae*, *Veillonellaceae* *Bacteroidaceae* *Enterobacteriaceae* *Enterococcaceae* *Lachnospiraceae* *Lactobacillaceae* *Ruminococcaceae*
Sun, 2017 [26]	SDI 4.25	SDI 4.34 Non-significant difference	*Verrucomicrobiales Synergistales*	*Clostridiaceae*
Kidney transplantation studies				
Fricke, 2014 [31]	OTU 301 SDI IQR 3–5	OTU 224 SDI IQR 2–5 Significant reduction	*Clostridiales*	*Lachnospiraceae*

IQR, interquartile range; OTU, operational taxonomic unit; SD, standard deviation; SDI, Shannon diversity index.

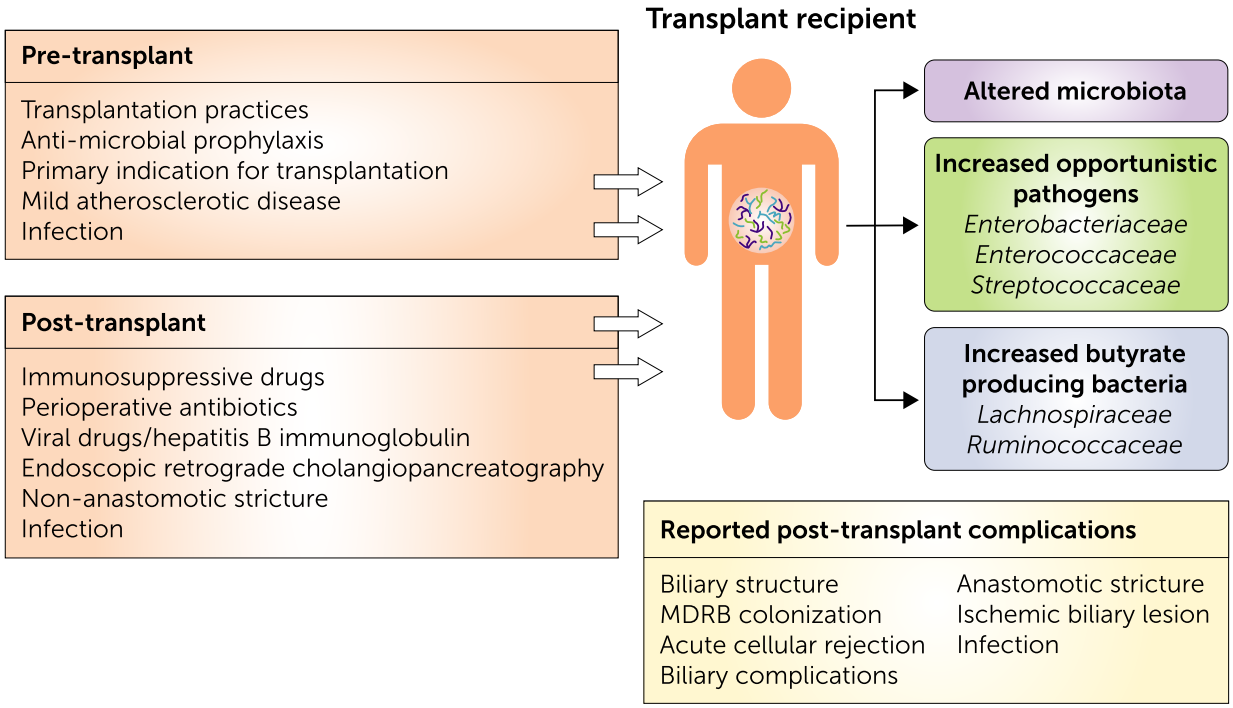

Figure 3 Schematic representation summarizing the factors contributing to enteric dysbiosis and post-transplant complications from the 13 included studies.

postoperative infections and diarrhea [31,33]. Our review identified two studies with decreased SDI post-transplantation, which was linked to postoperative infection, acute cellular rejections, and bloodstream infections. Initial post-transplant changes in the gut microbiome made a lasting impact on the compositional diversity as there was no significant changes between one and six months after transplant. [31]. Even though microbial diversity improves with time after SOT, Zhang *et al.* [30] still found the microbiome at 15–21 months post-transplant to be less diverse than healthy controls. The dissimilarity in the microbial diversity may be due to the difference in analysis timing; for example, Bajaj *et al.* noted the significant increase in microbial diversity 7 ± 3 months post-transplant, whereas Fricke *et al.* and Kato *et al.* noted significant decrease in the diversity 1 and 2 months post-transplant, respectively. Apart from the length of the microbial analysis, the compositional transformations of IM are highly variable due to the type of transplant and patient themselves along with surgical procedures, antibiotics, and IS. Additionally, end-stage diseases that necessitated SOT are themselves associated with enteric dysbiosis. We noted some of the pre-transplant families such as *Enterobacteriaceae*, *Enterococcaceae*, and *Ruminococcaceae* to be enriched in post-transplant period. A thorough comparison of pre-transplant taxa with post-transplant taxa was not feasible because not all studies included pre-transplant data.

Our systematic review is limited by the small number of clinical studies and population heterogeneity that contribute to difficulties in comparability. The antimicrobial, immunosuppressive drugs, and the timing of sample collection post-transplant differed among studies. Moreover, the study aims, methodology, and evaluated outcomes were variable and it was thus difficult to compare IM composition. Some studies reported a relative abundance of pre- and post-transplant microbiome, while others used the LDA or simply reported the most abundant taxa. Therefore, the included studies were analyzed based on the microbial presence of each order or family within each of the included studies. Functional analysis of the post-transplant microbiome could not be performed due to a lack of metagenomic and shotgun sequencing data or inferred functional metagenomic analysis of 16S rRNA sequencing data in the included studies.

Conclusion

Emerging evidence shows that there is a bi-directional relationship between the host and the intestinal microbiome, which is critical to health as well as pathogenesis and progression of the disease. Our systematic review

Transplant International 2020; 33: 1163–1176

provides insight into the changes that occur in the microbiome after transplant, with compositional changes of IM and predominance of pathogenic taxa. These microbial alterations may play a role in generating a higher risk of metabolic disease, malignancy, and infection post-transplant. Thus, more research is essential to determine whether changes in the composition and function of IM after SOT are causative or simply an association. Nonetheless, the types of taxonomic changes that occur in transplant recipients are suggestive of causation, given what is known in the non-transplant literature about the contribution of these taxa to metabolic disease, infection, and cancer. Further knowledge on whether the IM is causal in post-transplant complications would help in the development of preventive strategies such as modulating the microbiome with prebiotics, diet, and exercise.

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Conflicts of interest

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Search strategy.

Table S2. Summary of liver and kidney transplant studies.

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