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Kidney transplantation and gut microbiota

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ABSTRACT

Kidney transplantation is an effective way to improve the condition of patients with end-stage renal disease. However, maintaining long-term graft function and improving patient survival remain a key challenge after kidney transplantation. Dysbiosis of intestinal flora has been reported to be associated with complications in renal transplant recipients. The commensal microbiota plays an important role in the immunomodulation of the transplant recipient responses. However, several processes, such as the use of perioperative antibiotics and high-dose immunosuppressants in renal transplant recipients, can lead to gut dysbiosis and disrupt the interaction between the microbiota and the host immune responses, which in turn can lead to complications such as infection and rejection in organ recipients. In this review, we summarize and discuss the changes in intestinal flora and their influencing factors in patients after renal transplantation as well as the evidence related to the impact of intestinal dysbiosis on the prognosis of renal transplantation from *in vivo* and clinical studies, and conclude with a discussion of the use of microbial therapy in the transplant population. Hopefully, a deeper understanding of the function and composition of the microbiota in patients after renal transplantation may assist in the development of clinical strategies to restore a normal microbiota and facilitate the clinical management of grafts in the future.

Keywords: gut dysbiosis, immunosuppression, kidney transplantation, microbial therapy

INTRODUCTION

End-stage renal disease (ESRD) has long been one of the most common causes of death worldwide. Patients with ESRD require renal replacement therapy, such as long-term maintenance hemodialysis or peritoneal dialysis treatment. Kidney transplantation is one of the most effective treatments for ESRD [1]. Since the 1960s, kidney transplantation has been used to treat patients with ESRD, improving their quality of life, reducing the cost of treatment, and extending their life expectancy.

However, the high incidence of complications such as infection, graft rejection, and diabetes mellitus after transplantation is a persistent challenge. Recently, the distribution and composition of gut microorganisms have been reported to be associated with complications in renal transplant recipients [1]. Gut microbiota is a community of bacteria located in the gastrointestinal tract, with a density of up to 10^{11}–10^{12} microorganisms per millimeter in the colon. Metagenomic profiles of colonic mucosa-associated microbiota have revealed that most of the gut microbiota of healthy individuals consists of Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [2]. Renal transplant recipients undergo a series of processes in the perioperative period, including surgical stress, use of antibiotics and high-dose immunosuppressants,

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changes in the internal environment, dietary changes, and the use of acid-suppressing agents, which can affect the distribution and composition of gut microbes. As a result, the impact of intestinal dysbiosis on renal transplantation has attracted significant attention in the field of transplantation. In this review, we summarize and discuss the changes in intestinal flora and their influencing factors in patients after renal transplantation as well as the evidence related to the impact of intestinal dysbiosis on the prognosis of renal transplantation from *in vivo* and clinical studies, and conclude with a discussion of the use of microbial therapy in the transplant population. The search was conducted in the following electronic databases: MEDLINE, Embase, and PubMed. These electronic databases were searched on (until May 2024) using the following terms and keywords: (Gastrointestinal Microbiome) AND (Anti-Bacterial Agents), (Gastrointestinal Microbiome) AND (Immunosuppressive Agents OR Mycophenolic Acid OR Tacrolimus OR Prednisolone OR Cyclosporine OR Sirolimus OR Rapamycin OR Everolimus), (Kidney Transplantation) AND (Gastrointestinal Microbiome), and (Probiotics OR Prebiotics OR Fecal Microbiota Transplantation) AND (Kidney Transplantation).

GUT MICROBIOTA

The composition of the flora of a particular part of the digestive tract reflects the physiologic properties of that part of the tract [3]. The distribution of intestinal microorganisms along the gastrointestinal tract varies significantly due to differences in intestinal pH, mucosal thickness, and intestinal motility. From stomach to colon, the density of intestinal bacteria gradually increases as the oxygen content decreases, with the highest number of bacteria in the colon. These intestinal flora include not only beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, but also potentially pathogenic bacteria, such as *Escherichia coli* and *Aspergillus*. They form a dynamic equilibrium symbiosis with the human body, which plays an important role in maintaining immune homeostasis and resisting the invasion of foreign pathogens [4, 5].

Metagenomic profiles of colonic mucosa-associated microbiota have revealed that most of the gut microbiota of healthy individuals consists of Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [2]. The intestinal flora is inextricably linked to the host, and a variety of factors can lead to changes in the structure, quantity, and function of the intestinal flora. Intrinsic factors include the individual's genetic make-up, age, gender, body immune response, and disease state [6–9]. Extrinsic factors include diet, smoking, lack of physical activity, surgical procedures, early exposure to stress and adversity, living conditions, and changes in the global environment [6, 10–19]. In addition, it has been found that almost all common medications have a wide range of effects on the human gut flora, such as psychotropic drugs and immunosuppressants [20], with antimicrobials and proton pump inhibitors being the ones that have the greatest impact on the gut microbiota [9].

Impacts of antibiotics on intestinal flora

The impacts of antibiotics on the intestinal flora can be generally categorized into several broad patterns, including interference with the composition of the intestinal flora, such as inhibition of Actinobacteria phylum and amplification of Anaplasma phylum, as well as the promotion of antibiotic resistance, which severely disrupts short- and long-term microbial homeostasis [21].

As early as 2007, Jernberg *et al*. demonstrated the effects of antibiotics such as clarithromycin, clindamycin, metronidazole, and ciprofloxacin on the structure of microbiota [22]. Recently, Duan *et al*. showed that the use of β-lactam, glycopeptide, and macrolide antibiotics was associated with a decrease in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* spp. [23]. These results further elucidate the new pathogenesis of a series of possible diseases brought about by antibiotic-induced dysbiosis of the intestinal flora, and provide us with new therapeutic ideas. We can treat illnesses caused by antibiotic-induced dysbiosis of the intestinal flora by supplementing with probiotics. On the other hand, the use of antibiotics does not only change the composition of the intestinal flora, but also increases the susceptibility of the intestine to drug-resistant bacteria as a result [24]. Rebecca *et al*. noted that antibiotic use is associated with changes in intestinal resistance gene load in children and may influence the diversity of antibiotic resistance genes [25].

Interestingly, it has been suggested that antibiotic-induced dysbiosis of the gut flora is associated with the onset and progression of autoimmune diseases. Marcela *et al*. noted that alterations in the Firmicutes, Proteobacteria, and Bacteroidetes after antibiotic intervention led to worsening of autoimmune disease [26]. This provides new directions for the pathogenesis and therapeutic options for autoimmune diseases, such as IgA nephropathy. We can hopefully prevent, treat and even reverse the onset and progression of autoimmune diseases through microbial therapies (e.g. fecal microbial transplants, probiotics, etc.).

Impacts of immunosuppressants on intestinal flora

It is well known that renal transplant recipients need to take immunosuppressants for a long time to prevent rejection and maintain good graft function [27]. The impacts of immunosuppressants on intestinal flora after renal transplantation have been a hot topic of research. Tables 1 and 2 summarize the effects of immunosuppression on gut flora after kidney transplantation. However, most studies have been limited to animal experiments. We need to conduct more clinical studies to understand the effects of immunosuppressants on the gut flora of immunosuppressed populations.

In 2017, Zaza *et al*. proposed for the first time that there were specific differences in the inhibitory effects of different immunosuppressive agents on gut microbes in kidney transplant recipients [28]. They found that the abundance of starch and sucrose metabolism pathway genes was lower in fecal samples from renal transplant recipients receiving everolimus + mycophenolate mofetil (EVE + MMF) maintenance therapy than in the tacrolimus + mycophenolate mofetil (TAC + MMF) group, whereas the macrolide transport system mrsA (*msrA*) was significantly enriched in EVE + MMF. Meanwhile, flagellar motor switch protein (*fliNY*) and type IV pilus assembly protein pilM (*pilM*) were significantly increased in the gut microorganisms of the TAC + MMF group compared to the EVE + MMF group [28]. Such drug-induced changes in microbial metabolism may specifically affect intestinal habit and modify susceptibility to infections. However, little is still known about the effects of everolimus on intestinal flora. In fact, everolimus is minimally used in kidney transplant recipients compared to sirolimus.

Mycophenolate mofetil (MMF) is one of the most commonly used immunosuppressive agents in kidney transplant recipients. MMF use was found to lead to intestinal dysbiosis in mice, which was mainly characterized by an increase in the abundance of *Escherichia* spp. and *Shigella* spp. in the *proteobacteria* and

Table 1: Effects of immunosuppressants on intestinal flora (animal experiments).

Immunosuppressive agent	Increased microorganism concentrations	Decreased microorganism concentrations	Reference
MMF	pathogenic *E. coli*		Tourret *et al.* [29]
	Escherichia/Shigella	*Bacteroidetes*	Flannigan *et al.* [30]
	Escherichia/Shigella		Taylor *et al.* [31]
		Firmicutes *Bacteroidetes*	Jardou *et al.* [32]
	Lachnospiraceae NK4A136	*Lachnospiraceae UCG-001*	Llorenc *et al.* [42]
TAC	*Allobaculum* *Bacteroides* *Lactobacillus* *F. prausnitzii*	*Clostridium* *Ruminococcus* *Rikenella* *Oscillospira*	Zhang *et al.* [36]
		phylum Firmicutes family Lachnospiraceae genus *Coprococcus*	Han *et al.* [38]
Rapamycin	*Alistipes* *Allobaculum* *Bacteroides*	*Lachnospiraceae NK4A136* *Lachnospiraceae UCG-014* *Akkermansia*	Jiao *et al.* [37]
	segmented filamentous bacteria		Bitto *et al.* [43]
		Marinilabiliaceae	Jung *et al.* [44]
		Turicibacter *Roseburia* *Oscillospira* *Mollicutes* *Rothia* *Micrococcaceae* *Actinomycetales* *Staphylococcus*	Bhat *et al.* [45]
		Alphaproteobacteria	Schinaman *et al.* [46]
Prednisolone	*Proteobacteria*	*Akkermansia*	Han *et al.* [40]
	Firmicutes	Bacteroidetes *Clostridium sensu stricto genus*	Tourret *et al.* [29]
Combined immunosuppressive regimen[a]	*E. coli*	*Clostridium sensu stricto* genus	Tourret *et al.* [29]

[a]prednisolone + MMF + TAC.

Table 2: Effects of immunosuppressants on intestinal flora (clinical studies).

Immunosuppressive agent (*n* of studies)	Solid organ (*n* of studies)	Increased microorganism concentrations	Decreased microorganism concentrations	Outcomes	Reference
TAC ($n = 43$)	Kidney ($n = 19$)	*F. prausnitzii*		Increased tacrolimus dosing	Lee *et al.* [47]
	Heart ($n = 24$)	*Akkermansia* *Rumninococcaceae*		Higher endotoxemia, lower levels of inflammation and oxidative stress	Jennings *et al.* [48]

a decrease in the abundance of Firmicutes and Bacteroidetes [29–32]. Interestingly, the researchers found that MMF use altered the composition of the gut microbiota, selecting for bacteria expressing the enzyme β-glucuronidase (GUS), and that the presence of GUS enzymes that bind the flavin mononucleotide (FMN) is significantly correlated with efficient MPA reactivation, which induced colonic inflammation, diarrhea, and weight loss [31, 33]. Zhang *et al.* noted that among renal transplant recipients with post-transplant diarrhea, patients with higher fecal β-glucosidase activity had a longer duration of diarrhea ($\geq$7 days) compared to those with lower fecal β-glucuronidase activity [34]. Thus, fecal β-glucuronidase activity may be a new biomarker for

gastrointestinal-associated MMF toxicity. In addition, Shivank *et al*. noted that the use of broad-spectrum antibiotics prevented mycophenolate-induced gastrointestinal side effects in mouse experiments [35]. However, translating preclinical findings into clinical practice is not easy and we need more clinical studies to confirm this conclusion.

Tacrolimus (TAC) is the other immunosuppressant most commonly used in patients after kidney transplantation. Zhang *et al*. found that in mouse experiments, only the high-dose TAC-treated group (10 mg/kg·d) showed significant changes in the intestinal microbiota, whereas no such changes were observed in the medium-dose (1 mg/kg·d) and low-dose TAC-treated groups (0.1 mg/kg·d), which suggests that TAC exerts a dose-dependent effect on the intestinal microbiota of the mouse. This was characterized by an increase in the abundance of *bacilli* such as Bacteroidetes and a decrease in the abundance of *bacilli* such as *Clostridium difficile*. Interestingly, in the Firmicutes, the abundance of *Allobaculum* and *Lactobacillus* is increasing, whereas the abundance of *Clostridium* and *Ruminococcus* is decreasing. In addition, microbe-related metabolic activities such as protein synthesis and degradation, energy metabolism, carbohydrate metabolism, lipid metabolism, and xenobiotic degradation were also reduced in the high-dose TAC treatment group [36]. Notably, two short-chain fatty acids (SCFAs) (acetate and pyruvate) that significantly affect immune function were altered in the high-dose TAC-treated group. These two types of SCFA are known to induce FoxP3$^+$ Treg via binding to endogenous receptors and G protein-coupled receptor 43 as a histone deacetylase inhibitor, which leads to a significant increase in the levels of Treg in the colonic mucosa and mesenteric lymph nodes, as well as in the systemic circulation, significantly affecting immune function [36]. In another animal study, Jiao *et al*. found a significant increase in the relative abundance of *Alistipes*, *Allobaculum*, and *Bacteroides* in the TAC group and a significant decrease in the abundances of *Lachnospiraceae_NK4A136*, *UCG-014*, and *Akkermansia* as compared with the control group. Jiao *et al*. further found that the decreased abundances of *Lachnospiraceae_NK4A136* and *Akkermansia* after TACtherapy may lead to decreased butyrate levels and subsequent hyperglycemia [37]. *Akkermansia* is well known for its probiotic effects and plays an important role in mediating systemic inflammation by maintaining the integrity of the intestinal barrier and preventing translocation of neurotoxic metabolites. Meanwhile, it is also important in regulating tryptophan metabolism along the kynurenine pathway, a key metabolic pathway in the brain–gut microbiome axis. In addition, Han *et al*. found that antibiotic use enhanced the effects of TAC on the gut microbial community in mice, particularly on the metabolic functions of microorganisms primarily associated with lipid metabolism [38]. Overall, high-dose TAC treatment alters the gut microbiome and associated metabolic activities and exerts an important immunosuppressive role in the colonic and systemic immune response.

Steroids are one of the core immunosuppressive agents used in kidney transplant recipients. Tourret *et al*. found that after 14 days of treatment with prednisolone in mice, there was an increase in the number of Firmicutes in the feces, along with a decrease in Bacteroidetes. In addition, the abundance of *Clostridium sensu stricto* was significantly reduced in ileal samples from mice in the prednisolone group and the combined treatment group (prednisolone + MMF + TAC). Interestingly, they found a parallel relationship between the proportion of *Clostridium sensu stricto* in the ileum and the distribution of C-type lectins (e.g. Reg3β and Reg3γ) antimicrobial peptides (AMPs) secretion [29]. As an important pattern-recognition receptor in the innate immune system, the expression of C-type lectins is important in controlling the microbiota at intestinal mucosal surface. It has been previously shown that loss of Reg3γ is associated with increased abundance of segmented filamentous bacteria and *Eubacterium rectale* [39]. However, no experiments have been performed to demonstrate whether the decreased abundance of *Clostridium sensu stricto* is a cause or a consequence of alterations in innate defense. Gram-positive bacteria are usually susceptible to Reg3β and Reg3γ; therefore, Tourret *et al*. suggest that the decrease in *Clostridium* after IS drug treatment is more likely to be a response to altered bacterial competition [29].

Mammalian target of rapamycin (mTOR) inhibitors are a commonly used class of immunosuppressants in patients after kidney transplantation. An animal study found that the clinical-mimicking dose of sirolimus reduced the thickness of the intestinal mucosal layer, increased the intestinal permeability, and enriched the circulating pro-inflammatory factors, including interleukin (IL)-12, IL-6, monocyte chemotactic protein 1, granulocyte-macrophage colony stimulating factor, and IL-1β [40]. We need more research to confirm this finding.

To date, most studies have shown that the use of immunosuppressive agents reduces the variety and abundance of gut flora, increases intestinal permeability and promotes inflammation, leads to disturbances in the intestinal flora and reduces microbe-associated metabolic activity, significantly affects immune function, and increases susceptibility to infections, which can then lead to a range of disorders such as inflammation of the colon and weight loss. Interestingly, however, an animal study found that cyclosporine use increased the abundance and diversity of gut flora, thereby ameliorating liver transplantation injury and partially restoring the gut microbiota after allogeneic liver transplantation, which also suggests that immunosuppression may also be beneficial to gut homeostasis [41].

However, clinical studies on the effects of immunosuppressive agents on the intestinal flora after organ transplantation are scarce. More representative are the studies by Lee *et al*. on the effect of TAC on the intestinal flora after renal transplantation and Jennings *et al*. on the effect of TAC on the intestinal flora after heart transplantation (Table 2).

Lee *et al*. characterized the fecal microbiota in the early period after renal transplantation by 16S rRNA deep sequencing. They found *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* are the most common phyla [47]. To the best of our knowledge, this is the first description of the gut flora in the early period after renal transplantation. Lee *et al*. found that the relative abundance of *Faecalibacterium prausnitzii*, which can produce large amounts of butyrate, was significantly higher in the Dose Escalation Group than in the Dose Stable Group during the first week after transplantation. Thus, the drug absorption and/or metabolism of TAC may be directly related to a healthy colonic mucosa that requires butyrate. In addition, a functionally healthy gut microbiota may influence TAC metabolism via CYP3A4 and P-glycoprotein in intestinal epithelial cells, which may explain the positive correlation between *F. prausnitzii* abundance and TAC dose [47]. However, this study did not delve into the potential mechanisms by which *F. prausnitzii* affects TAC metabolism and the small sample sizes of this study reduced the certainty of the findings. Interestingly, Lee *et al*. later found that *F. prausnitzii* can produce a unique TAC metabolite, suggesting that intestinal bacterial metabolism is a previously unrecognized pathway for drug elimination [47].

In addition, Jennings *et al*. found that several potentially anti-inflammatory taxa, including the genera *Akkermansia* and the family Rumninococcaceae, were increased in the TAC high-dose group during the early period after heart transplantation (within

the first 3 months), which was associated with lower levels of biomarkers of inflammation and oxidative stress, as well as higher endotoxemia [48]. However, due to the relatively small sample size of the study, these findings were only marginally statistically significant. Second, this was a cross-sectional analysis, and longitudinal trends in the relationship between gut microbiota dysbiosis and TAC dose requirements were not assessed. Although the central hypothesis of this study is that the gut flora influences the metabolism of TAC, it is possible that this drug may influence the composition of the gut flora. Third, patients in this study were not genotyped for CYP3A5 polymorphisms, which can significantly alter the pharmacokinetics of TAC. Fourth, dietary habits were not assessed in the included patients, so we could not determine whether dietary habits contributed to the gut microbiota profile observed in this study. In future studies, dietary assessment should be included.

In conclusion, there are few clinical studies on the effects of immunosuppressants on the intestinal flora of patients after renal transplantation. We need more clinical studies to explore the effects of immunosuppressants on the intestinal flora of immunosuppressed populations, so as to better guide clinical treatment and alleviate/reduce complications after renal transplantation.

CHANGES IN INTESTINAL FLORA IN PATIENTS AFTER RENAL TRANSPLANTATION

Renal transplant recipients undergo a series of processes in the perioperative period, including surgical stress, use of antibiotics and high-dose immunosuppressants, changes in the internal environment, dietary changes, and use of acid-suppressing agents, all of which can affect the distribution and composition of intestinal microorganisms [49]. To date, several studies have reported changes in gut flora after solid organ transplantation, including kidney transplantation. The intestinal flora after transplantation is characterized by a reduction in diversity, a decrease in the abundance of baseline dominant flora, and the emergence of new dominant flora, which often implies an increased risk of infection. Table 3 summarizes the changes in intestinal flora of patients after kidney transplantation in different studies.

Guirong *et al*. analyzed the gut flora of kidney transplant recipients, chronic kidney disease (CKD) patients, and healthy individuals in comparison and found that kidney transplant recipients had the lowest levels of microbial enrichment. Compared to healthy individuals, renal transplant recipients and CKD patients had decreased abundance of *Lachnospiraceae*, *Ruminococcaceae*, and *Faecalibacterium*, and significantly increased abundance of *Bacteroidetes*, *Proteobacteria*, *Clostridiales*, and *Enterobacteriaceae*. Interestingly, contrary to the results of most studies, they reported a decrease rather than an increase in the abundance of *Firmicutes* in kidney transplant recipients [50]. In another study, Chan *et al*. categorized *Firmicutes* into two types, and they reported that the abundance of *Firmicutes_A* was significantly higher and increased after renal transplantation, whereas the abundance of *Firmicutes_G* was relatively low and decreased significantly after transplantation [51]. This may explain the conflicting results in different studies. Xiao *et al*. in a review on organ transplantation and intestinal flora emphasized that increased abundance of pathogenic proteobacteria may be responsible for the development of infections after transplantation [52]. Moreover, it has been previously shown that increased abundance of proteobacteria can serve as a marker for ecological dysbiosis or disorders of the intestinal flora [53].

When Lee *et al*. compared the gut microbiota of patients with and without diarrhea, they found decreased abundances of *Eubacterium*, *Anaerostipes*, Coprococcus, *Romboutsia*, *Ruminococcus*, *Dorea*, *Faecalibacterium*, *Fusicatenibacter*, *Oscillibacter*, *Ruminiclostridium*, *Blautia*, *Bifidobacterium*, and *Bacteroides* and an increase in *Enterococcus*, *Escherichia*, and *Lachnoclostridium* in patients with diarrhea. Most those differences were independent from antibiotic use and time after transplantation [54]. In another study, Westblade *et al*. reported that ~ 30% of kidney transplant recipients carried at least one diarrhea pathogen, most commonly *C. difficile*. In addition, as measured by the Chao1 index, they found that kidney transplant recipients carrying *C. difficile* had reduced microbial diversity [55]. These data suggest that colonization of pathogenic gut bacteria increases and protective gut bacteria decreases after kidney transplantation, increasing the risk of infectious complications such as urinary tract infections (UTIs) and diarrhea. However, they did not find a significant relationship between detection of gastrointestinal pathogens, including *C. difficile*, and the development of post-transplant diarrhea. By contrast, only a small number of subjects who were colonized by gastrointestinal pathogens at the time of transplantation developed post-transplant diarrhea, and of these, few had the same pathogens at the time of transplantation and at the time of post-transplant diarrhea, underscoring the importance of detecting the etiology of post-transplant diarrhea infections in kidney transplant recipients. In addition, they found that *C. difficile* toxins A&B were not detected by the toxin immunoassay in most cases [55]. Given the established view that *C. difficile* infection is a toxin-mediated disease, these data suggest that most patients did not have active *C. difficile* infection at the time of transplantation.

In conclusion, an imbalance between pathogenic and protective microbiota characterizes the ecological dysbiosis of the gut flora in renal transplantation, and this dysbiosis may be mainly related to the intensive use of immunosuppressive and antimicrobial drugs (Table 3). However, the etiology behind the altered diversity of the gut microbiota after kidney transplantation cannot be accurately determined because of the multiple and complex processes involved in transplantation. Ischemia-reperfusion injury, the alloimmune response, and the use of immunosuppressive agents and antimicrobials are potential factors for altered gut microbiota characteristics. However, due to the complexity of the process and the associated ethical issues, finding the most critical factor behind the altered gut flora in human subjects is nearly impossible.

IMPACTS OF INTESTINAL FLORA DYSBIOSIS ON THE OUTCOME OF KIDNEY TRANSPLANTATION

Studies have shown that the microbiota of an individual could alter the immune response of organ transplantation hosts via specific signaling pathways, such as the Myd88 and TLR9 pathways, enabling successful transplantation [61]. However, conventional therapies (e.g. immunosuppressive drugs and prophylactic antibiotics given after transplantation) may dysregulate the intestinal microbiota, leading to certain complications after transplantation such as the risk of infection (urinary infections and infectious diarrhea), adverse immune phenomena (autoimmune hemolytic anemia), transplant rejection, and increased mortality. Moreover, when postoperative

Table 3: Changes in intestinal flora in patients after renal transplantation.

Reference	Country/ Area	Year	N	Immunosuppressive regimen	Time after surgery	Increased microorganism concentrations	Decreased microorganism concentrations	Outcomes
Fricke *et al.* [56]	USA	2014	60 KTRs	–	1 month after transplantation	*Firmicutes* *Bacteroides* *Actinobacteria* *Proteobacteria*	*Anaerotruncus* *Coprobacillus* *Coprococcus* *Peptostreptococcaceae*	–
Zaza *et al.* [28]	Italy	2017	20 KTRs	9(EVE + MMF + methylprednisolone) 11(TAC + MMF + methylprednisolone)	at least 6 months after transplantation	*Firmicutes* *Proteobacteria* *Actinobacteria*		↑ msrA (EVE + MMF) ↑ fliNY, pilM (TAC + MMF)
Guirong *et al.* [50]	China	2018	16 KTRs/ 84 CKD/ 53 healthy	–	1 month after transplantation	*Bacteroidetes* *Proteobacteria* *Clostridiales* *Enterobacteriaceae*	*Firmicutes* *Lachnospiraceae* *Ruminococcaceae* *Faecalibacterium*	↑Metabolism of (carbohydrates/amino acids/xenobiotics) ↓Metabolism of (cofactors/vitamins/ nucleotides/terpenoids)
Lee *et al.* [57]	USA	2019a	168 KTRs/ 54healthy	–	in the first 3 months after transplantation		*F. prausnitzii* *Holdemanella biformis* *Eubacterium dolichum* *Coprococcus comes* *Subdoligranulum variabile* *Eubacterium rectale*	↓Butyrate-producing gut microbiota
Lee *et al.* [54]	USA	2019b	71 KTRs	–	in the first 3 months after transplantation	*Firmicutes* *Proteobacteria*	*Bacteroides*	Post-transplant diarrhea
Westblade *et al.* [55]	USA	2019	142 KTRs	–	in the first 10 days after transplantation	*Proteobacteria* *Firmicutes* *Bacteroides*		–
Swarte *et al.* [58]	The Nether-lands	2020	139 KTRs/ 105 healthy	25(18%, Cyc) 79(57%, TAC) 13(9%, Aza) 100(72%, MMF) 133(96%, PSL)	at least one year after transplantation	*Firmicutes* *Proteobacteria*	*Actinobacteria*	↓ Butyrate-producing bacteria
Yu *et al.* [59]	China	2021	10 KTRs	–	1 month after transplantation	*Bacilli* *Enterococcaceae* *Enterococcus*		–
Chan *et al.* [51]	Australia	2021	12 KTRs/ 12donors	TAC + MMF + PSL	1∼2 months after transplantation	*Firmicutes_A* *Roseburia spp.*	*Firmicutes_G*	↓Microbial richness and Shannon diversity scores in kidney transplant recipients
Ma *et al.* [60]	China	2021	28 KTRs/ 30healthy	TAC + Aza + PSL	—	*Proteobacteria* *Bacteroidetes* *Enterococcus* *Escherichia_Shigella* *Rothia* *Streptococcus*	*Firmicutes(Clostridiaceae)* *Faecalibacterium* *Prevotella_9* *Blautia* *Ruminococcus* *Agathobacter* *Subdoligranulu*	↓Butyrate-producing bacteria ↓Carbohydrate utilization, the degradation of indigestible dietary carbohydrates and host carbohydrates ↑UTI (*Enterococcus*)

KTRs, kidney transplant recipients; EVE, everolimus; Aza, azathioprine; PSL, prednisolone; Cyc, cyclosporin.

Table 4: Pathogenic and protective microbiota in the gut microbiota after kidney transplantation.

Pathogenic microbiota	Protective microbiota	Ambiguous microbiota	Other/unspecified microbiota
C. difficile [63]	*Akkermansia* [66]	*Firmicutes* [36, 63]	*Anaerostipes*
E. coli [64]	*Bacteroidetes* [67]		*Blautia*
E. faecalis [63]	*B. pseudocatenulatum* [65]		*Coprococcus*
Lactobacillus [65, 66]	*Clostridia and Clostridiales* [68]		*Dorea*
Proteobacteria [52, 53]	*[Eubacterium] rectale* [65]		*Fusicatenibacter*
Streptococcus [63]	*F. prausnitzii* [65]		*Oscillibacter*
Verrucomicrobium [63]	*Lachnospiraceae NK4A136* [37]		*Ruminococcaceae*
	Romboutsia [64]		

complications occur, microecological dysregulation is more pronounced [62].

Dysbiosis of intestinal flora plays a crucial role in the outcome of organ transplantation, especially renal transplantation. Wu *et al*. concluded that *Verrucomicrobium*, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* are the four most abundant bacterial species in the intestinal tract of patients after renal transplantation, and that these bacterial species can affect solid organ transplantation. For example, some bacteria belonging to *Firmicutes* (e.g. *C. difficile*, *Enterococcus faecalis*, and *Streptococcus*) can infect solid organ transplant recipients, and are an important cause of side effects (diarrhea, bloodstream infections, and pneumonia) after solid organ transplantation. Compared to kidney transplant recipients without diarrhea, those with diarrhea had lower abundance of *Bacteroides*. Proteus composed of a variety of Gram-negative bacteria (such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli*) is a risk factor for increased infection, bacteremia, and mortality in solid organ transplant recipients [63]. Table 4 summarizes the pathogenic and protective microbiota, as well as the ambiguous microbiota, in the gut flora after renal transplantation mentioned in this review.

Infection

The altered gut microbiota, especially the abundance of pathogenic bacteria, increases the risk of post-transplant infectious complications such as diarrhea, UTIs, respiratory tract infections, and resulting graft rejection. Lee *et al*. reported in a study on diarrhea after kidney transplantation that decreased abundances of *Eubacterium*, *Anaerostipes*, *Coprococcus*, *Romboutsia*, *Ruminococcus*, *Dorea*, *Faecalibacterium*, *Fusicatenibacter*, *Oscillibacter*, *Ruminiclostridium*, *Blautia*, *Bifidobacterium*, and *Bacteroides* and an increase in *Enterococcus*, *Escherichia*, and *Lachnoclostridium* in patients with diarrhea. Surprisingly, they reported an association between the decreased abundance of the diversity of gut commensals, not an increase in gut pathogen colonization [54]. Another study reported that ~30% of post-renal transplant subjects carried at least one diarrheal pathogen, most commonly *C. difficile* [55]. In addition, Swarte *et al*. found a relationship between a decrease in butyric acid-producing bacteria and post-transplant diarrhea [58].

UTs are one of the most common infections in kidney transplant recipients. Huang *et al*. summarized the role of the gut microbiota in the development of UTIs. The abundance of *E. coli* in the intestinal flora was independently associated with the development of *E. coli* UTIs and, in some patients, the *E. coli* strains in the urine were most similar to those in the intestines, supporting the idea that intestinal bacteria are the source of UTIs. In addition, SCFAs produced by *Faecalibacterium* and *Rombousia* have been shown to inhibit the growth of *Enterobacteriaceae*, and increased abundance of intestinal *Faecaliberium* and *Romboutsia* was significantly associated with reduced risk of *Enterobacteriaceae* UTI. This suggests that SCFA-producing gut bacteria may reduce the risk of *Enterobacteriaceae* UTIs by inhibiting the growth of *Enterobacteriaceae* in the gut [64]. Another study by Lee of 168 kidney transplant recipients also found that a relative abundance of butyric acid-producing bacteria <1% in the gut microbiota increased the risk of respiratory viral infections that could lead to graft rejection, independent of BKV and CMV viremia [57]. This reinforces the important role of SCFA-producing bacteria in preventing infections.

It has previously been shown that the composition of the gut microbiota is very similar between individuals living in the same environment [69]. Besides, dietary habits also affect the composition of the gut microbiota [15]. In a study by Kim *et al*., most living unrelated donors were spousal donors. These spousal donors who lived in the same environment were in close contact during their lives, and shared meals more frequently. Kim *et al*. found that the gut microbial communities of spousal donors were very similarly characterized and that kidney transplant recipients had a lower incidence of infections in the 6 months following transplantation [70]. These data suggest that pre-transplant microbial similarity between unrelated donors and recipients may be related to 6-month allograft function, which provides some new references for matching kidney transplant donors and recipients.

Inflammation

Ardalan *et al*. summarized a bidirectional relationship between inflammation and gut dysbiosis. Ischemia-reperfusion and immunosuppression expose transplant recipients to inflammation, which in turn may affect the microbiota; and dysbiosis of the intestinal flora may cause changes in the intestinal immune system, such as intestinal inflammation, increased intestinal permeability, and impaired tolerance to food/microbial antigens [62].

A leaky gut barrier leads to activation of the NF-kB pathway, dysregulation of the immune response, and chronic production of pro-inflammatory cytokines, resulting in systemic inflammation. When gut microbiota are disturbed, inflammatory reactions can be activated via the NF-KB pathway, reducing the degree of cresol and IL-4 in intestinal mucosa, and increasing the degree of urea nitrogen, which will lead to kidney damage [1]. Moreover, innate immune resetting can enhance graft inflammation and activate alloreactive T cells (microbial antigen's

specific memory T cells) that may cross-react with donor major histocompatibility complex antigens and provoke graft rejection or block tolerance induction [62].

The use of antibiotics has long been recognized as an influential factor in intestinal flora disorders. Interestingly, Wu *et al*. showed that ampicillin pretreatment could inhibit the infiltration of inflammatory cells such as monocytes, macrophages, and neutrophils after renal transplantation by modulating the ratio of intestinal flora in mice, and significantly reduced the expression of inflammatory factors such as TNF-α, IFN-γ, IL-6, and IL-1β in the grafts of renal transplanted mice to reduce renal injury [63].

These findings provide a basis for the existence of the gut-immune axis *in vivo*. Based on these findings, a method to re-establish intestinal flora and stabilize intestinal microecology could be developed for innovative use in the prevention and treatment of kidney diseases.

Rejection

Wang *et al*. characterized the gut microbiota composition of patients with antibody-mediated rejection (AMR) of kidney allografts, with the most significant alteration being a decrease in *Clostridia* and *Clostridiales* in the AMR group [68]. Li *et al*. found that a significant decrease in species richness was detected in the AMR group, mainly in the form of a decrease in the Chao 1 and ACE indices, while no differences in microbiota community diversity were observed between the two groups because changes in the Shannon and Simpson indices were not significant. They found that the relative abundance of *F. prausnitzii*, *[Eubacterium] rectale*, *[Ruminococcus] torques*, *Coprococcus catus*, and *Bifidobacterium pseudocatenulatum* were decreased in recipients with AMR. *F. prausnitzii* is the most important butyrate-producing bacteria in human colon. Generally, *F. prausnitzii* play an anti-inflammatory role by producing butyrate and salicylic acid and inducing IL-10. Similarly, *[Eubacterium] rectale* and *B. pseudocatenulatum* could help to maintain intestinal barrier and suppress inflammation activation through inhibiting CD83 and TLR4/NF-κB, respectively. Moreover, increased *Lactobacillus* counts were observed in patients with CKD and recipients with AMR, with increases predominantly in *Lactobacillus fermentum*, *Lactobacillus johnsonii*, and *Lactobacillus acidophilus*. All of these have been shown to have enhanced immune responses, especially antibody response, leading to the development of AMR [65].

Li *et al*. further found that the fecal metabolome also changed significantly in AMR patients compared to controls, and that these specific differences in fecal bacterial species and metabolites were strongly correlated with clinical indicators of AMR and could be used as a diagnostic biomarker to differentiate between renal transplant recipients suffering from AMR and those with stable renal function. Notably, the combined model with both microbial and metabolic markers had an AUC >0.9, suggesting that it may have high diagnostic value for AMR [65]. Easily accessible fecal samples and improvements in multi-omics technology will enable microbiota-based diagnosis in recipients with AMR. This non-invasive diagnostic approach will reduce or even replace the standard invasive approach (renal biopsy) to differentiate renal transplant recipients with AMR from those with stable renal function. In addition, Li *et al*. hypothesized that changes in the structure and function of the gut microbiota may lead to alterations in the fecal metabolite taurocholate, which may affect the pathogenesis and progression of AMR, but this hypothesis needs to be substantiated by further clinically randomized studies, which will be important for understanding the exact role of the gut microbiota in AMR.

The effect of gut microbiota on tolerance of allogeneic grafts is also one of the research hotspots. Wu *et al*. found that a high-fiber diet prevented intestinal dysbiosis after allogeneic kidney transplantation in mice compared to mice fed normal food. High-fiber diet mice had a higher abundance of *Bifidobacterium* spp., *Bacteroides* spp., and *Clostridiales* spp., which can produce SCFAs. Then, SCFAs promote Treg cell development through the GPR43 receptor. Mice on a high-fiber diet exhibited better grafted function on days 14 and 100 after allogeneic kidney transplantation. Graft survival was prolonged and rejection was reduced. Interestingly, allograft mice receiving supplemental sodium acetate showed similar protection against rejection and subsequently exhibited donor-specific tolerance. In contrast, mice deficient in $CD25^+$ Tregs or mice deficient in the SCFA receptor GPR43 did not exhibit this anti-rejection protection [67]. Dietary therapies that induce changes in the gut microbiome can alter the immune system in mice and deserve to be investigated as a potential clinical strategy to promote transplantation tolerance and, hopefully, reduce the use of immunosuppressive drugs.

New-onset diabetes after transplantation

New-onset diabetes after transplantation (NODAT) is a common complication after kidney transplantation and is strongly associated with mortality and graft loss in recipients. As many as 30% of kidney transplant recipients may develop NODAT. Currently, the first step in preventing NODAT is to assess and eliminate risk factors in patients at high risk for diabetes after transplantation. There is growing evidence that impaired glucose metabolism is associated with gut microbiota composition. A study by Lecronier *et al*. found an association between the gut microbiota after kidney transplantation and NODAT. They found that the relative abundance of *Lactobacillus* sp. detected in NODAT patients after kidney transplantation was higher than that in controls, while the proportion of *Akkermansia muciniphila* in NODAT patients was 2500-fold lower than that in controls. Therefore, they speculated that the presence of *Lactobacillus* sp. and the lack of *A. muciniphila* may be risk factors for NODAT [66]. In addition, a recent study comprehensively analyzed the gut microbiota of patients with NODAT: the abundance of *proteobacteria* decreased while the abundance of *Bacteroides* increased. Besides, they also found that the biosynthesis of unsaturated fatty acids was strongly associated with NODAT. The number of bacteria producing SCFAs decreased, while the number of pathogenic bacteria increased [71]. Therefore, a decrease in the number of SCFA-producing flora may be a risk factor for NODAT.

Diabetes is a common side effect of TAC. NODAT was reported to occur in 33.6% of organ recipients on TAC, which was higher than the percentage observed in patients on cyclosporine A (26.0%) [72]. However, in some cases it is difficult to eliminate these risk factors, as the use of TAC or cyclosporine is essential in many cases. Jiao *et al*. found that TAC altered the composition of the intestinal microbiota in mice (Table 1), decreased the concentration of butyric acid in the cecum, and significantly increased FBG, HbA1c, and OGTT levels in mice. By contrast, oral butyrate supplementation restored butyrate concentrations in the cecum after TAC treatment and restored FBG, HbA1c, and OGTT to normal levels [37]. It has been previously shown that butyric acid produced by gut microbiota stimulates insulin secretion by binding to the membrane receptor GPR41/43 to induce GLP-1 and PYY production by colonic L cells, thereby

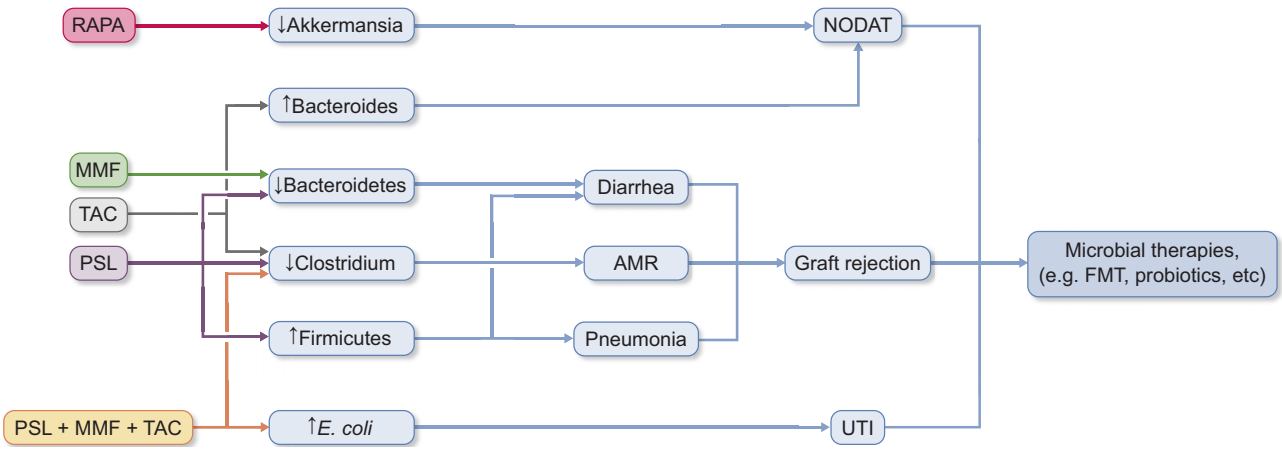

Figure 1: Immunosuppressants that may lead to certain changes in the gastrointestinal microbiota and the subsequent impacts on the outcome of kidney transplantation. RAPA, rapamycin; TAC, tacrolimus; MMF, mycophenolate mofetil; PSL, prednisolone.

improving glucose homeostasis [73]. Jiao *et al*. found that butyric acid levels were significantly reduced after TAC treatment. Besides, although the expression of GPR43 was not changed in the intestinal mucosa, the expression of GPR43 in the crypts was significantly decreased. After butyric acid supplementation, the expression of GPR43 in the crypts increased significantly [37]. TAC treatment resulted in a significant decrease in GLP-1 expression in the colon and a decrease in serum levels of GLP-1/PYY, which led to a decrease in insulin secretion from pancreatic β-cells. Butyrate supplementation successfully reversed TAC-induced hyperglycemia by regulating GLP-1, PYY, and insulin. These results indicate that TAC-induced hyperglycemia is caused by reduced levels of butyrate in the colon, and that blood glucose can be lowered by oral supplementation with butyrate, which regulates GLP-1 and PYY levels through the 'butyrate-GPR43-GLP-1' pathway in the intestine. Therefore, Jiao *et al*. propose a new clinical strategy in which supplementation with butyrate or its dietary precursors can complement the reduction of butyrate by TAC treatment without discontinuing TAC, thus minimizing the risk of hyperglycemia and preventing NODAT [37]. These findings suggest that alterations in the gut microbiota and SCFAs after transplantation are independently associated with diabetic status in patients after kidney transplantation. The abundance of fecal *Lactobacillus* sp. and *A. muciniphila* may be used as predictive markers to assess the risk of NODAT.

Hypertension

Another common complication after renal transplantation is hypertension, which is often caused by TAC. Toral *et al*. suggest that TAC-induced hypertension may be mediated by intestinal microecological dysregulation. In this study, mice on TAC had lower intestinal flora diversity, increased *Firmicutes/Bacteroidetes* and lower SCFA production. Meanwhile, mice in the TAC group had a higher degree of vascular oxidative stress and an altered Th17/Treg mesenteric balance. Further studies found that mice supplemented with *Lactobacillus fermentum* CECT5716 via fecal microbial transplantation partially reversed vascular abnormalities [74].

These studies suggest that gut ecological dysregulation from various causes after kidney transplantation negatively affects transplantation outcomes (Fig. 1). Changes in the relative abundance of certain bacterial species in the gut may be used as predictors of poor outcomes, particularly graft rejection and post-transplant infections. These post-transplant ecological disorders and complications are associated with poor allograft outcomes and increased mortality in transplant recipients. Since there is no anatomical link between the kidney and the gastrointestinal tract, the mechanisms involved in intestinal microecological dysregulation affecting the kidney should be further investigated to explain these phenomena.

MICROBIAL THERAPIES

Microbial therapies can change the composition of the intestinal flora, reduce the adverse effects caused by the imbalance of the flora, protect the intestinal barrier, strengthen the function of the immune system, and inhibit the invasion of pathogenic microorganisms. Microbial therapies, including probiotic and prebiotic supplementation and fecal microbial transplantation, have great potential to reduce the incidence and/or severity of various human diseases. Studies have shown that microbial therapies may have a positive effect on antibiotic-associated diarrhea, irritable bowel syndrome, inflammatory bowel disease, atopic eczema, necrotizing small intestinal colitis, and systemic metabolic disorders (e.g. obesity and type II diabetes mellitus) [75]. In recent years, more and more studies have applied microbial therapies to the transplant population by correcting intestinal flora dysbiosis and thus reducing/mitigating post-transplant complications.

Probiotics

Organ transplant recipients need to take immunosuppressants for a long time after surgery to prevent rejection and maintain good graft function, and this greatly increases the risk of post-operative infections in transplant recipients. Therefore, in the field of solid organ transplantation, probiotics were first applied to prevent infection. Zhang *et al*. found that liver transplant recipients supplemented with probiotics had a reduction in the prevalence of bacterial infections from 30% in the placebo group to 8% in the intervention group [76]. For kidney transplant recipients, *Lactobacillus* supplementation not only prevent and treat diarrhea, but also prevents *C. difficile* infections while receiving antibiotics and immunosuppressant therapy [77].

Table 5: Administration of probiotics in SOT in humans.

Organ transplantation	N	Probiotic administration	Results	Ref.
Liver	66	Lactic acid bacteria (*Pediacoccus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei* ssp. paracasei F19, *L. plantarum* 2362)	↓Bacterial infection rates	Rayes *et al.* [80]
Liver	67	*Bifidobacterium lactis*, *L. Plantarum*, *L. Acidophilus*, *L. Rhamnosus*, *L. Casei*, *L. Brevis*	↓Incidence of bacterial infections ↓Duration of antibiotic therapy	Zhang *et al.* [76]
Kidney	36	*L.s plantarum*, *L. casei* subsp. *rhamnosus*, *L. gasseri*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *L. acidophilus*, *L. salivarius*, *L. sporogenes*, *Streptococcus thermophilus*	↓Concentration of plasma p-Cresol	Guida *et al.* [78]
Kidney	34	*L. plantarum* 299v (LP299v)	↓Incidence of *CDI*	Dudzicz *et al.* [77]
Kidney	24	the combination of *L. plantarum* and *L. paracasei*	↑Incidence of decreasing creatinine ↑the estimated glomerular filtration rate (eGFR) ↑a trend of trough levels of tacrolimus and sirolimus	Chan *et al.* [79]

In addition, in the field of kidney transplantation, researchers have found that probiotic supplementation can improve kidney function and facilitate the removal of toxins from the body of kidney transplant recipients. Guida *et al.* found that dysbiosis of intestinal flora in renal transplant recipients increases the production of p-Cresol, along with a decreased clearance of p-Cresol by the transplanted kidney, and therefore, this uremic toxin accumulates in kidney transplant recipients. In this study, plasma p-Cresol levels decreased by 33% from baseline after 30 days of probiotic treatment in the intervention group, while plasma p-Cresol levels remained stable in the placebo group, and there were no significant changes in liver and kidney function in either group. This indicates that probiotic treatment can promote the clearance of plasma p-Cresol in renal transplant recipients while ensuring normal hepatic and renal function [78]. Besides, Chan *et al.* found the incidence of decreasing creatinine gets higher (odds ratio 13.3, 95% CI 1.64–77.2, $P = .01$) in transplant recipients using *Lactobacillus plantarum* and *Lactobacillus paracasei* (Lm), which was demonstrated by a decrease in creatinine by 0.06 mg/dl ($P = .02$) and an increase in glomerular filtration rate by 3.1 ml/min/1.73 m^2 ($P = .03$) after Lm supplementation. Furthermore, this study showed a trend of higher trough levels of TAC and sirolimus after Lm supplementation, which might provide a potential strategy for reducing the dosages of immunosuppressants [79]. Researchers have emphasized the role and outcome of probiotics in complications after solid organ transplantation (Table 5).

Prebiotics

Prebiotics are food components that selectively promote the growth of beneficial gut bacteria, and their likely mechanism of action in the body is to increase SCFA production and lower intestinal pH. Supplementation of prebiotics not only promotes the growth of probiotics such as *Bifidobacterium* and *Lactobacillus* in renal transplant recipients, but also reduces plasma uremic toxin levels, increases the levels of SCFAs, and promotes the secretion of glucagon-like peptide 1 (GLP-1), which is beneficial to the control of body weight, diabetes mellitus, and high blood pressure [78].

A randomized controlled study of 56 kidney transplant recipients showed that participants experienced a significant reduction in gastrointestinal symptoms during the 7 weeks of prebiotic intervention [−0.28 (interquartile range, IQR −0.67 to 0.08) vs −0.07 (IQR −0.27 to 0), $P = .03$], but the control and intervention groups were similar in infectious events (33% versus 34%, $P = .83$), including bacteremia, UTIs, and respiratory tract infections [81]. While this study suggests that prebiotics can significantly reduce gastrointestinal symptoms, however, this study has some of its limitations. First, gastrointestinal symptoms in this study were measured using the Gastrointestinal Symptom Rating Scale score between baseline and the end of the study but did not specify what the symptoms were. Second, this trial was limited by the small sample size and relatively short study duration, which limited meaningful analysis of certain outcomes (e.g. infections).

In addition, a recent study found that kidney transplant recipients in the prebiotic group experienced less abdominal pain (34% vs. 59%, $P = .03$) and reflux (36% vs. 55%, $P = .04$) compared to the placebo group after 6–8 weeks of prebiotic supplementation while gastrointestinal symptoms were similar in both groups at baseline in terms of abdominal pain (65% vs. 68%, respectively, $P = .39$) and reflux (58% vs. 61%, $P = .56$). Also, subjects assigned to the intervention group had lower microbial richness and Shannon diversity scores at baseline compared to the placebo group. However, after 6–8 weeks of prebiotic supplementation, microbial richness (from 96 ± 46.5 to 142 ± 38.6, $P = .01$) and Shannon diversity (from 2.87 ± 0.49 to 3.42 ± 0.52, $P = .06$) were significantly increased in the intervention group, whereas these metrics did not change in the placebo group [82]. The main strengths of this study are its randomized design and the provision of both taxonomic and functional understanding of the gastrointestinal microbiota. However, these strengths need to be balanced against limitations, including its small sample

size, which limits statistical power and the accuracy of effect estimates.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is the transfer of feces from a healthy donor into the colon of a patient with a disease caused by an altered microbiota, with the goal of restoring a normal microbiota and thereby curing the disease. The earliest application of FMT was in the treatment of recurrent *C. difficile* infection (CDI). The mechanism of applying FMT for the treatment of CDI is thought to be the reconstitution of the intestinal microbiota by diminishing the metabolic niche acquired by *C. difficile* in the ecology of the patient's colon. The microbial ecology of a patient's colon after FMT shows increased diversity and reconstitution of phyla that promote colonic health, including *Firmicutes*, *Bacteroidetes*, and *Faecalibacterium*, in which healthy and diverse commensal flora effectively colonize the intestinal lumen and mucosa, preventing competition or coexistence of pathogenic microorganisms [83]. Various formulations and routes of administration have been investigated: nasoduodenal tube, oral capsule, or most typically the liquid form during colonoscopy. Although a recent trial demonstrated that oral capsules were noninferior, the lower gastrointestinal delivery modalities, colonoscopy, has been reported to be more effective [84].

Treatment of CDI-induced diarrhea

C. difficile, an anaerobic Gram-positive, spore-forming bacillus, has become the most common cause of nosocomial infectious diarrhea and has been associated with increased mortality in all populations. Patients who have undergone solid organ transplantation (SOT) are at increased risk of CDI and recurrent CDI (rCDI), which may be associated with chronic immunosuppression, frequent antibiotic use, and prolonged hospitalization. Traditional treatments include vancomycin and fidaxomicin. Over the past decade, FMT has not only become an effective and safe alternative for the treatment of CDI and rCDI in the general population, but has also made significant progress in the field of SOT.

A multicenter, retrospective study demonstrated that FMT is safe and effective in the treatment of recurrent, severe, or fulminant *C. difficile* infections [85]. Common adverse events include diarrhea, cramping, belching, nausea, abdominal pain, bloating, and transient fever, with deaths occurring primarily in critically ill patients or older adults with serious comorbidities [84]. Lin *et al*. concluded that antibiotic use is a major driver of CDI for most patients, with cephalosporins being a common trigger. In some patients, prophylactic Bactrim may also increase the risk of CDI [84]. Therefore, the risks and benefits of antimicrobial use should be weighed in patients with risk factors for CDI, especially post-FMT patients, to minimize recurrence rates. In addition, candidates for FMT should be carefully selected. We need to keep in mind the major comorbidities of FMT, risk factors for recurrence after FMT and risks involved with certain FMT delivery modalities.

Of note, the majority of rCDI patients selected for FMT were in the late post-SOT period (>6 months) and were in a state of stable immunosuppression. Thus, a limitation of this study is that we cannot specifically comment on the safety or efficacy of FMT in patients who are in the early post-SOT or who have recently enhanced immunosuppression. Larger prospective studies are needed before guidelines for the treatment of CDI associated with FMT can be changed to include patients with SOT.

Treatment of urinary tract infections

In recent years, in addition to being used in the treatment of rCDI, more and more studies have shown the feasibility of FMT in the treatment of UTIs. Biehl *et al*. applied FMT to treat one renal transplant recipient with recurrent UTI (rUTI) but without CDI. To our knowledge, this is the first report of application of FMT to treat a renal transplant recipient with rUTI but without CDI. The most recent UTI of this patient before FMT was caused by *E. coli*. Microbiota analysis of the patient's urine samples after application of FMT treatment showed a significant decrease in *E. coli* over time [86]. However, random physiological variations in relative abundance of *E. coli* cannot be ruled out due to the only one set of specimens available for analysis. Therefore, further studies are needed to investigate the potential interactions between the gut and urinary tract microbiota in rUTIs. This case report suggests new ideas on how to deal with similar cases in the future. Patients with refractory UTIs are usually exposed to long-term antibiotic prophylaxis with associated risks of adverse events or development of antibiotic resistance, ultimately leading to untreatable UTIs. In renal transplant recipients, long-term use of immunosuppressive agents further increases the risk of UTIs, and FMT appears to significantly improve the health status of such patients. Therefore, we will continue to support the decision to apply FMT to cases of refractory rUTIs.

In summary, clinical evidence suggests that supplementation of prebiotics and probiotics, FMT may alter gut microbiota composition and reduce infection complications. However, the limited number, size, and duration of these studies means that the efficacy and safety of these measures in renal transplant recipients remain uncertain, and therefore they are not currently suitable for generalization in routine clinical practice. Further well-designed large clinical trials are necessary. Another consideration is that if microbial therapies are found to be effective and safe, the administration of additional medications to renal transplant recipients would need to be carefully considered given the significant medication burden issues that this population already faces.

CONCLUSIONS

Kidney transplantation is an effective treatment for ESRD. However, post-transplant complications such as infections and rejection remain its main challenges. All these post-transplant complications can affect the success of the transplant and can even be life-threatening. Studies have shown a correlation between the composition of gut flora and events such as graft rejection, UTIs, and diarrhea in patients after kidney transplantation. In addition, gut microbiota composition plays an important role in metabolic complications and viral infections after transplantation. There is still much to be researched about the role of gut flora and its derived metabolites in graft function and complications after renal transplantation.

There is a growing interest in the bidirectional relationship between the gut microbiota and kidney transplant complications. Organ transplantation leads to dysbiosis of the gut flora, which can cause the development and progression of complications in kidney transplant recipients. Moreover, changes in the diversity of the gut microbiota after kidney transplantation are

more pronounced at the onset of post-transplant complications. Studies to date have shown that intestinal flora diversity may be influenced by a variety of factors. In addition to surgical stress and antibiotic use, the use of immunosuppressive drugs after kidney transplantation has a dramatic impact on the intestinal flora, leading not only to an increase in pathogenic bacteria and a decrease in protective bacteria, but also to changes in various metabolic activities, such as a decrease in SCFA caused by a decrease in SCFA-producing bacteria, which increases the risk of post-transplant complications. For example, there is a relationship between a decrease in butyric acid-producing bacteria and post-transplant diarrhea, UTIs, and respiratory tract infections.

Dysbiosis of the intestinal flora may cause changes in the intestinal immune system, such as intestinal inflammation and increased intestinal permeability. A leaky gut barrier leads to activation of the NF-kB pathway, dysregulation of the immune response, and chronic production of pro-inflammatory cytokines, which further leads to systemic inflammation, renal damage and even triggers graft rejection. In the gut microbiota of patients with antibody-mediated allogeneic renal transplant rejection (AMR), we found a decrease in the relative abundance of SCFA-producing bacteria, such as *F. prausnitzii*, which may potentiate the role of the donor-specific antibody response, leading to AMR. In addition, changes in the structure and function of the gut microbiota may lead to alterations in the fecal metabolite taurocholate, which may affect the pathogenesis and progression of AMR, but this hypothesis needs to be substantiated by further clinically randomized studies. Further studies found that a high-fiber diet may prevent rejection and improve tolerance to grafts by increasing the abundance of SCFA-producing bacteria in the intestinal flora of mice. Dietary therapies that induce changes in the gut microbiome can alter the immune system in mice and deserve to be investigated as a potential clinical strategy to promote transplantation tolerance and, hopefully, reduce the use of immunosuppressive drugs.

NODAT is a common complication after renal transplantation, and up to 30% of renal transplant recipients may develop NODAT. Studies have shown that NODAT occurs in 33.6% of organ recipients on TAC. There is growing evidence of an association between the gut microbiota after kidney transplantation and NODAT, and the presence of *Lactobacillus* sp. and the lack of SCFA-producing bacteria such as *A. muciniphila* may be risk factors for NODAT. Further studies found that TAC-induced hyperglycemia could be successfully reversed by supplementing mice with butyrate. Therefore, we hypothesized that supplementation with butyrate or its dietary precursor could complement the reduction in butyrate from TAC treatment without discontinuing TAC, thereby minimizing the risk of hyperglycemia and preventing NODAT.

Since the development and progression of many post-transplant complications are influenced by the microbiota, modulation of the gut microbial community may be an important approach to improve long-term graft survival. Increasing attention is being paid to the modulation of gut flora in kidney transplant recipients, such as probiotics, prebiotics, and fecal microbial transplants. In recent years, an increasing number of studies have applied microbial therapies to the transplant population to correct intestinal dysbiosis, thereby reducing/mitigating post-transplantation complications, such as preventing infections, alleviating gastrointestinal symptoms, and controlling NODAT. Further research to expand knowledge in this area and to address many of the ill-defined areas is key to future research.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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