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Study of Gut Microbiota Changes in Liver Transplantation Patients

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ABSTRACT

Liver transplantation (LT) is a single option for treatment end-stage of liver diseases. Although repeated antibiotic therapy can cause major changes in the intestinal microbiota. There is growing evidence regarding the role of gut microbiota in the before and after operative course and their impact on patient outcomes in LT. It plays a crucial role in controlling the process of digestion because gut microbiota produce, extract, and absorb a wide range of metabolites, such as lipids, vitamins, bile acids, short chain fatty acids, and amino acids. Additionally, gut microbiota have the ability to directly stop the colonization of foreign bacteria by impeding their growth by appropriating available resources and/or producing antibacterial molecules. This study was conducted on 31 liver transplant patients in Gastroenterology Surgical Center (GEC), Mansoura University, Egypt. We collected 87 stool samples for the current study, dividing them into 3 groups, before LT, after one week from LT and two weeks from LT. Isolated bacteria from patient before LT including Phylum: Bacteroidota (38.1%), phylum: Firmicutes (58.75%) and Phylum: Proteobacteria (3.17%). Isolated bacteria from patients after one week of LT including Phylum: Bacteroidota (38.1%), Phylum: Firmicutes (58.7%) and Phylum: Proteobacteria (39.0%). Isolated bacteria from patients after two weeks LT including Phylum: Bacteroidota (21.43%), Phylum: Firmicutes (58.7%) and Phylum: Proteobacteria (35.7%).

Key Words:

Liver transplantation, Microbiota, Bacteroidota, Firmicutes, Proteobacteria.

1. INTRODUCTION

Liver transplantation (LT) is the main therapeutic choice for patients with end-stage liver [1]. With an estimated $>10^{13}$ cells in the mammalian gastrointestinal lot, commensal microbes play a significant part in the support of stable gut physiology and organism homeostasis. Not only is the gut microbiota essential

for food substrate digestion, absorption, and storage, but it is also required for host immune system development, particularly in terms of maintaining gut immune system homeostasis [2]. Furthermore, good immunological homeostasis can promote or inhibit the proliferation of both beneficial and harmful microbes [3]. The human digestive tract is home to a diverse and intricate community of microorganisms known as the gut microbiome. As a result of antibiotic therapy, interventions, altered anatomy from surgery, biliary complications and immunosuppression, end-stage liver disease, such as LT, is frequently associated with changes in the composition of the gut microbiome [4-6]. Microorganisms that dwell in the gastrointestinal tract [3] are called fecal microbiota. Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria are the most well-known bacteria in the gastrointestinal tract [3], as per stool example [7]. Infection risk in LT beneficiaries changes over time [8]. Typically, transplant procedures and nosocomial infections are to blame for patients' difficulties in the early post-transplant period [9]. Between one and twelve months after transplantation, opportunistic infections become increasingly common because to the mounting load of immunosuppression [10]. Conversely, patients of transplants continue to be susceptible to community-acquired infections, and those who experience persistent allograft failure or recurrent cholestatic liver disease may experience recurrent cholangitis [11]. When faecal enema was used to treat four patients with pseudomembranous colitis in 1958, Faecal microbita transplant (FMT) was first applied in modern medicine [12]. Recurrent *Clostridium difficile* infections (CDI) have been involved to FMT, and there is also emerging evidence linking it to autoimmune diseases, several allergy conditions, inflammatory bowel disease, and metabolic disorders like obesity. FMT is a simple approach. Hospital-acquired infections (HAIs), also referred to as nosocomial infections (NIs), are infections that occur within hospitals [13]. A hospital, nursing home, rehabilitation centre, outpatient clinic, or other therapeutic institution may be the source of this kind of infection [14]. In the clinical setting, there are several methods that an infection can spread to vulnerable patients. Most often, healthcare personnel, tainted equipment, or interventional techniques (such as bed linens or air droplets) are the means of transmission. The infection may have come from the environment, another sick patient, or, in rare circumstances, it may not have had a known source [15]. Approximately 10% of patients experience postoperative surgical site infection after receiving a LT, making it one of the most common bacterial infections**.**

The goal of this research was to identify gut bacteria and modifications in the gut microbiota composition brought on by immune suppression, antibiotic therapy, surgically altered anatomy, and other interventions. And detect pathogenic bacteria as result to opportunistic infections and nosocomial infections.

2. METHODS AND MATERIALS

This study included 31 LT patients from Gastroenterology Surgical Center (GEC), Mansoura university, between March 2021 and March 2023. All individuals provided informed consent and were fully informed about the diagnostic methods involved and the nature of the condition. The study procedure followed the Helsinki Declaration 2013 ethical guidelines and was approved by the research ethics council of Faculty of Medicine, Mansoura University (IRB: MDP.23.08.131.R1). Before taking part in the study, all patients provided written informed consent. They were separated into three groups: one week before LT (n=31), one week after LT (n=28) and two weeks after LT (n=28). Three patients data were missing one week after and two week after LT surgery because exit immediately before the surgery based on vision of doctors. A total of 87 stool samples were collected from all patients. All specimens were transported to the microbiology laboratory in 5 ml sterile Amies transport media [16] at 4^{o}C within 2 hours. Following that cultivated on Brucella broth medium [17], Columbia blood agar base medium[18], MacConkey's Agar [19] and nutritional agar [19]. VITEK 2 compact 15 (Biomerieux, France) was used to identify the isolated bacteria visually and biochemically.

3. RESULTS and DISCUSION

Commensal bacteria are important participants in the maintenance of stable gut physiology and organismal homeostasis, with an estimated $>10^{13}$ cells in the mammalian gastrointestinal tract. The gut microbiota is not only required for food substrate digestion, absorption, and storage, but also for host immune system development, particularly in terms of controlling gut immune system homeostasis [2]. Furthermore, proper immunological homeostasis may stimulate or limit the proliferation of both helpful and dangerous microorganisms [3]. This work aimed to identify microbiota in LT patients, This study was

conducted on 31 LT patients. Stool samples were taken from each patient within 3 weeks (one week before, one week after and two week after surgery). The total stool samples for all patients were 87 samples.

In our study, the majority of LT patients were males with a percentage of 80.6% and females their percentage was 19.4%. It was also noted that patients under the age of 30 years were 9.7% and the percentage of patients between the ages of 30 to 40 years was 12.9%, while for patients between the ages of 40 to 50 years, the percentage was 19.3% and in patients between the ages 50 to 60 years was 48.4% and finally patients over 60 years of age recorded 9.7%, Fig (1). Our findings agreed with [20] reported that the percentage of male and female (79% and 21%, respectively). Also, according to [21] the percentage of male and female (60% and 40%, respectively). [22] also demonstrated that male and female patients had a percentage (70% and 30%, respectively). Also, [23] showed that male and female patients have percentage (87.5% and 12.5%, respectively), Ages ranging from ≥65 years 26 (12.0%) and <65 years 190 (88.0%). Additionally, [24] he explained in his study that included adults and children 70.4% male and 29.6% female patients. The median age at transplantation was 52 (interquartile range, 43–58) years. The recipients were divided into four age groups: Twenty years (11.9%), 20-39 years (8.6%), 40-59 years (63.3%), and 60 years (16.1%).We collected 87 stool samples in total for the current study, isolated bacteria before LT including, Phylum: *Bacteroidota (38.1%) (Bacteroides akkermansia, Bacteroides stercoris, Bacteroides melaninogenicus and Fusobacterium mortiferum). Phylum: Firmicutes (58.7%) (Clostridium difficile, Peptococcus, Ruminococcaceae, faecalibacterium prausnitzii, Enterococcus faecalis, Enterococcus gallinarum, Enterococcus faecium and Lactobacillus) and Phylum: Proteobacteria (3.17%) (Escherichia coli). Phylum: Bacteroidota (24.39%) (Bacteroides akkermansia, Bacteroides melaninogenicus and Fusobacterium mortiferum) were isolated from patients after one week of LT. Phylum: Actinobacteriota (4.88%) (Bifidobacterium breve). Phylum: Firmicutes (31.7%) (Clostridium, Peptococcus, Ruminococcaceae, Faecalibacterium prausnitzii, Enterococcus faecalis, Enterococcus gallinarum and Enterococcus faecium) and Phylum: Proteobacteria (39%). Phylum: Bacteroidota (21.43%) (Bacteroides akkermansia and Fusobacterium mortiferum) were isolated from patients after two weeks of LT. Phylum: Firmicutes (42.8%) (Clostridium difficile, Peptococcus, Ruminococcaceae, faecalibacterium prausnitzii, Enterococcus faecalis, Enterococcus faecium and Lactobacillus), Phylum: Proteobacteria (35.7%) (Escherichia coli, Acinetobacter baumannii, Enterobacter aerogenes, Enterobacter cloaecae, Enterobacter hormaechei, Klebsiella pneumoniae and Klebsiella oxytoca), Table (1).*

Our findings corroborated those of [25] showed that the patients fecal microbiota on the phylum level was notable for relatively diminished proportions of Firmicutes (52%) and Bacteroidetes (29%), whereas the percentage of Proteobacteria was dramatically increased (16%). Within the family of Proteobacteria, molecular fingerprinting detected the presence of many pathobionts including *Klebsiella pneumoniae, Enterobacter spp.* and *Acinetobacter baumannii*. [26] also found an increase in *Enterobacteriaceae* and *Enterococcaceae* species and a decrease in *Faecalibacterium prausnitzii* and *Bacteriodes*, as well as a decrease in overall gut microbiota diversity following liver transplantation. Furthermore, [27] discovered a significant decrease in the abundance of certain microbial species, such as *Actinobacillus* and *Escherichia coli*, as well as an increase in the abundance of other microbial species, such as Micromonosporaceae, Desulfobacterales, Sarcina genus of Eubacteriaceae, and *Bacteriodes akkermansia*. As well, [28] shown that following LT, there are fewer *Bifidobacterium* and *Lactobacillus* in the feces, but Enterobacteriaceae and *Enterococcus* are higher in rats that did not undergo a transplant. Additionally, [7],[29] demonstrated that the prominent gutmicrobial phylum are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with Firmicutes and Bacteroidetes accounting for 90% of the gut microbiota. Bacteroidetes is mostly composed of the species Bacteroides, which is frequently isolated from clinical specimens and is responsible for a variety of pathological diseases. Firmicutes, on the other hand, is represented by a huge variety of genera, including Gram-positive and Gram-negative bacteria. [30] reported that liver transplantations are complicated by infection, with bacterial infection being the most common and reported to range from 20-80% of all cases, Specific to bacteremia, higher levels of *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus gallinarum* have been implicated. Furthermore, research by [31],[32] demonstrated that Proteobacteria grows and Firmicutes declines in phylum level. Ruminococcus and the Enterobacteriaceae family have increased in LT patients, but the prevalence of bacteria from the Clostridium family has also changed, with declines in Roseburia and Faecali bacteria. Also, [33] demonstrated that patients following

LT were linked to a decrease in Firmicutes and Bacteroidetes and an increase in Proteobacteria at the phylum level, with an imbalance in the proportions between dominant families, such as Bacteroidaceae, and potentially pathogenic families, such as Streptococcaceae, Enterococcaceae, and Pseudomonadaceae. Additionally, [34] found that, in comparison to healthy individuals, post-LT patients had a significantly decreased gut microbial diversity, whereby the number of Firmicutes and Bacteroidetes at the phylum level is decreasing and Proteobacteria and Verrucomicrobia increasing. Intestinal dominating bacteria, such as Firmicutes and Bacteroidetes, are essential for preserving the intestinal homeostasis of the host. A reduction in these two bacteria was always a sign of compromised gut barrier integrity and a higher chance of bacterial translocation.

4. CONCLUSION

This study indicated that the gut microbiota composition of LT patients alters one week before one week after and two week after surgery. These changes in bacterial composition include a rise in potentially dangerous bacteria (e.g., Enterococcaceae and Enterobacteriaceae which include *Escherichia coli* and *Klebsiella*) and a decrease in dominating phylum bacteria (e.g., Bacteroidota and Firmicutes).

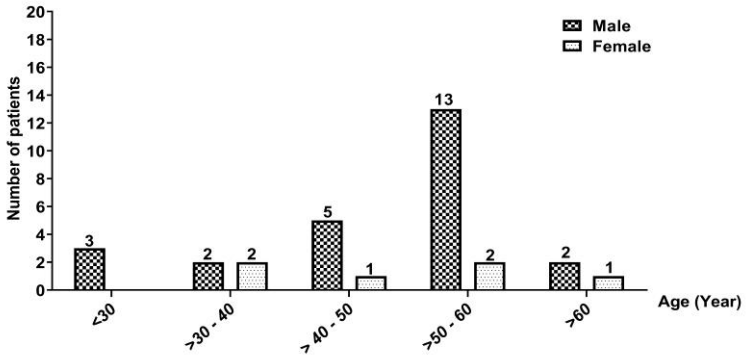


Fig. 1. Relationship between male and female according to age of LT patients.

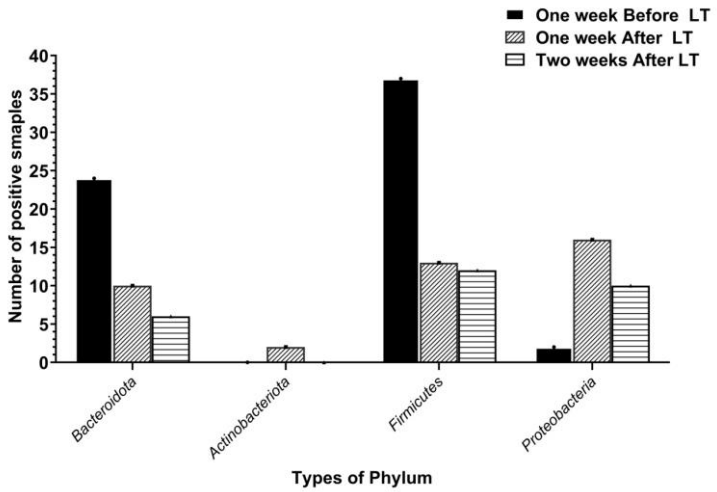
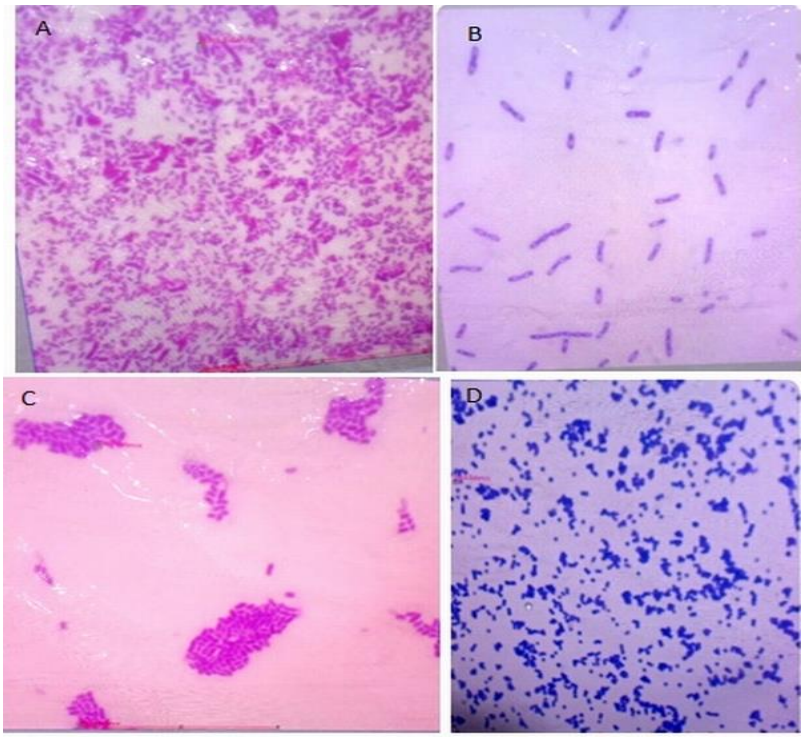


Fig 2. Isolated phylum of bacteria from stool cultures of LT patients .

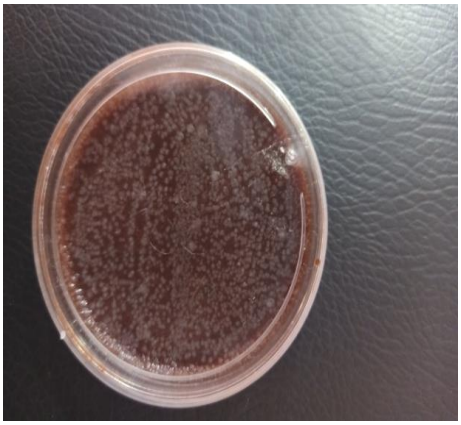
Fig 3. Examples of bacteria which identified on VITEK 2.



A. *Klebsiella pneumoniae*, B. *Colistridium difficile*, C. *Acinetobacter baumannii* and D. *Enterococcus faecium*

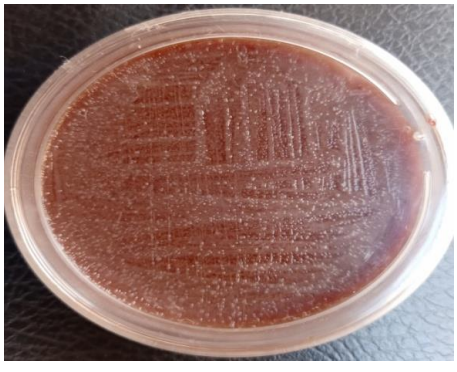
Fig 4. Examples of bacterial growth on different media.

Colistridium difficile on Columbia Blood Agar Base



Bacteroides akkermansia on Columbia Chocolate Agar Base.

Klebsiella pneumoniae on MacConkey's Agar.



Escherichia coli on Columbia Chocolate Agar Base.

Table 1. Isolated bacteria from stool cultures of LT patients.

Isolated bacteria	One week Before LT (n=63)	One week After LT (n=41)	Two weeks After LT (n=28)	Total (n=132)
Phylum: Bacteroidota				
Bacteroides akkermansia	8	5	3	16 (12.1%)
Bacteroides stercoris	4	0	0	4 (3%)
Bacteroides melaninogenicus	2	1	0	3 (2.27%)
Fusobacterium mortiferum	10	4	3	17 (12.9%)
Total	**24 (38.1%)**	**10 (24.39%)**	**6 (21.43%)**	**40 (30.3%)**
Phylum: Actinobacteriota				
Bifidobacterium breve	0 **(0%)**	2 **(4.88%)**	0 **(0%)**	2 **(1.5%)**
Phylum: Firmicutes				
Clostridium difficile	7	2	1	10 (7.6%)
Peptococcus	6	1	2	9 (6.8%)
Ruminococcaceae	9	4	4	17 (12.9%)
Faecalibacterium prausnitzii	4	1	2	7 (5.3%)
Enterococcus faecalis	3	2	1	6 (4.5%)
Enterococcus gallinarum	2	1	0	3 (2.27%)
Enterococcus faecium	4	2	1	7 (5.3%)
Lactobacillus	2	0	1	3 (2.27%)
Total	**37 (58.7%)**	**13 (31.7%)**	**12 (42.8%)**	**62 (47%)**
Phylum: Proteobacteria				
Escherichia coli	2	3	2	7 (5.3%)
Acinetobacter baumannii	0	2	1	3 (2.27%)
Enterobacter aerogenes	0	3	1	4 (3.0%)
Enterobacter cloaecae	0	2	2	4 (3.0%)
Enterobacter hormaechei	0	1	1	2 (1.5%)
Klebsiella pneumonia	0	3	2	5 (3.78%)
Klebsiella oxytoca	0	2	1	3 (2.27%)
Total	**2 (3.17%)**	**16 (39%)**	**10 (35.7%)**	**28 (21.2%)**

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