Association between Gut Colonization of Vancomycin-resistant Enterococci and Liver Transplant Outcomes

by

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ABSTRACT

Background: Vancomycin-resistant Enterococci (VRE) colonization is common in liver transplant candidates. In addition to the risk of invasive enterococcal infections, dominance of VRE colonization in the gut may contribute to low microbiota diversity playing a role in the transplant outcomes. The purpose of this study is to evaluate the association between VRE colonization and liver transplant on 6-month post-transplant complications and mortality at 2-years.

Methods: We performed a retrospective cohort analysis of all adult patients (≥18 years old) who underwent liver transplantation for chronic liver disease between 1st September 2014 and 31st December 2017 at the University of Alberta Hospital in Edmonton, Alberta, Canada. Health clinical outcome included patient and graft survival status, follow-up, and causes of death. The primary cause of death was used to calculate Kaplan-Meier survival analysis. Multivariate Analyses was performed to identify independent variables associated with outcome using Cox-regression Hazard Model. We calculated the hazard ratio at 95% confidence intervals of mortality and acute kidney injury at 30 days. Patient mortality was the primary endpoint. Acute rejection, clinically significant infections, ischemia reperfusion injury and acute kidney injury were secondary endpoints.

Results: Of the included 343 liver transplants, 67% were males with a median age of 56.5. The prevalence of VRE colonization pre-liver transplant was 19.8 % (68/343). VRE colonized patients had higher MELD scores pre-transplant than non-colonized patients (median MELD 24 vs 17; $p<0.001$), but other variables were similar between both groups. The association of VRE colonization with pre-defined endpoints was: acute kidney injury at 30 days (66% vs 54%, $p=0.066$), clinically significant bacterial/fungal infection (31% vs 21%, $p=0.074$), acute rejection

(12% vs 11%, p=0.779) and death (15% vs 11%, p=0.435). Eight patients had VRE infection: 3 VRE colonized and 5 non-colonized pre-transplantation. 27 patients without VRE colonization at baseline acquired VRE post-transplant (27/275, 9.8%). Probability of survival at baseline between the VRE colonized and the non-VRE colonized was p=0.215. Percentage-free of acute kidney injury at baseline was log rank test p=0.009 at 30 days. Of the 68 VRE colonized patients at baseline, there were 45 (66.2%) presenting AKI versus 144 (52.4%) non-AKI. VRE colonized had a higher hazard ratio (1.610, 95% CI: 1.127-2.299; p=0.009) for acute kidney injury at 30 days post-transplantation. Of the 95 VRE colonized patients at baseline death 12 (12.6%) versus 248 alive 17 (6.9%), the VRE colonized showed a trend towards high risk of mortality at 2-years after transplantation (1.974, 95% CI: 0.890-4.378; p=0.094).

Conclusion: VRE colonization pre-transplant was associated with the development of acute kidney injury and a trend towards high risk of mortality. VRE colonization is an independent predictor of complication in the liver transplant than MELD. These results suggest optimizing the management of these patients in the peri-transplant period, including renal-protective strategies in VRE positive patients. Further efforts are needed to decolonize patients before liver transplantation.

PREFACE

This thesis is an original work by Diana Alejandra Chiang Jurado. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Impact of Vancomycin-resistant Enterococci Colonization in Liver Transplant Patient-Outcome.", No. Pro00082528, May 29th, 2018.

The technical apparatus referred to in chapter 3 was designed by myself, with the assistance of Drs. C. Cervera Alvarez and J. Gonzalez-Abraldes. The data analysis in chapter 4 and concluding analysis in chapter 5 are my original work as well as chapter 1 and parts of chapter 2. The first half of chapter 2 on the topic of Vancomycin-resistant Enterococci of this thesis has been published as a review article under the authorship of Belga S, Chiang D, Kabbani D, Abraldes JG, Cervera C. The direct and indirect effects of vancomycin-resistant enterococci colonization in liver transplant candidates and recipients. *Expert Rev Anti Infect Ther*. 2019;17(5):363–373. doi:10.1080/14787210.2019.1607297.

DEDICATION

TO MOM AND DAD, CECILIA AND COLÓN
"LAS PALABRAS NUNCA
ALCANZAN CUANDO LO
QUE HAY QUE DECIR
DESBORDA EL ALMA"
-JULIO CORTÁZAR

TO MY LITTLE BROTHER, COLÓN
"MERECES LO QUE SUEÑAS"
-GUSTAVO CERATI

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LIST OF ABBREVIATIONS

ACDV Atherosclerotic cardiovascular disease

AKI Acute Kidney Injury

ALD Alcoholic Liver Disease

AMPK Activated protein kinase

AST Aspartate aminotransferase

BAL Bronchoalveolar lavage

BSI Blood stream infection

CMV Cytomegalovirus

CRD Chronic Renal Disease

DNA Deoxyribonucleic acid

Foxp3 Forkhead box P3

GPR 41, 43, 109a G-protein coupled receptor

HDAC Histone deacetylase

HDACi Inhibitors of Histone deacetylase

ICU Intensive Care Unit

IECs Intestinal epithelial cells

IQR Interquartile range

LTx Liver Transplant

MAMPs Microbial Associated Molecular Patterns

MDR Multidrug Resistant Bacteria

MELD Model of End Stage Liver Disease

NASH Non-alcoholic steatohepatitis

NF-kB Nuclear factor kappa-light-chain-enhancer of activated B cells

OLT Orthotopic Liver Transplant

OTTR Organ Transplant Tracking Record

pTreg T-regulatory cells

Reg3b Regenerating islet-derived protein 3-beta

RegIIIα Regenerating islet-derived 3-alpha

RegIIIϒ Regenerating islet-derived 3-gamma

RNA Ribonucleic acid

SBP Spontaneous bacterial peritonitis

SCFAs Short-Chain Fatty Acids

SOT Solid Organ Transplant

Th17 T helper 17 cells

Th1 T helper 1 cells

TLR Toll-like receptors

TNF^{delta} Tumor necrosis factor-delta

TMA Trimethylamine

TMAO Trimethylamine oxidase

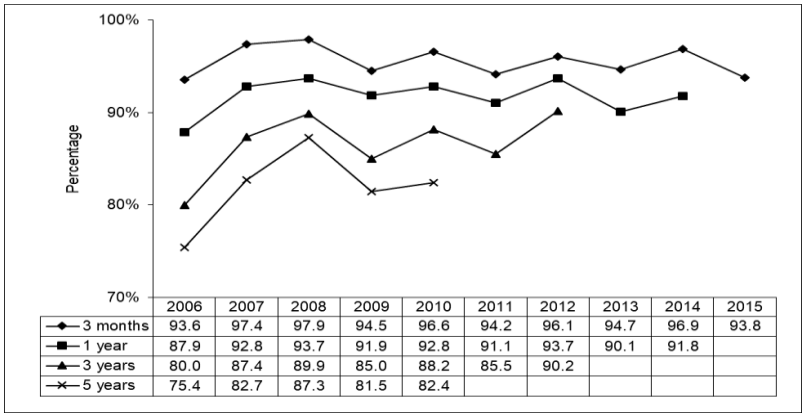
VRE Vancomycin-resistant Enterococci

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO LIVER TRANSPLANTATION AND VRE

Liver transplantation is currently the only curative therapeutic intervention for a number of liver diseases, such as end-stage liver cirrhosis, hepatocellular carcinoma and some metabolopathies, and then, in spite of the advances in surgical techniques, immunosuppression and prophylaxis therapy, there are a subset of transplant patients with complications and high mortality rate. Before patients proceed to receive a liver transplantation, they undergo a selection process according to the likelihood of optimal post-transplant survival. Although liver transplant survival rate within one year is 80%[1], around 15% of liver transplant recipients in Canada do not survive at 3-years post-transplant[2] (Figure 1). Importantly, one reason behind the high mortality rate in liver transplant recipients is attributed to early mortality within the first 6-months post-transplantation.



	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
3 months	93.6	97.4	97.9	94.5	96.6	94.2	96.1	94.7	96.9	93.8
1 year	87.9	92.8	93.7	91.9	92.8	91.1	93.7	90.1	91.8	
3 years	80.0	87.4	89.9	85.0	88.2	85.5	90.2			
5 years	75.4	82.7	87.3	81.5	82.4					

Figure 1. Unadjusted 3-month and 1-, 3-, 5-year patient survival rates for deceased-donor liver transplant recipients, first graft, Canada (excluding Quebec), 2006 to 2015.[2]

Fatal outcomes post-liver transplant is multifactorial. Liver patients share well-defined risk factors for fatal outcomes, such as previous hospital admissions, antibiotic use, predisposition to infections, surgical complications, among others. Classical risk factors in liver transplant are associated with early mortality post-transplantation and include: The Model for End-Stage Liver Disease (MELD) score, comorbidities, prophylactic antibiotic use, infections and post-transplant acute kidney injury. In patients with end-stage liver disease, risks factors are highly prevalent because they require prolonged hospitalizations, including intensive care unit

(ICU) admissions and exposure to broad-spectrum antibiotics. Seeing that, end-stage liver disease patients are placed at a major risk for colonization with antibiotic resistant bacteria. The number of patients attending consults due to liver diseases in North America is high, for example, in the US, there is an estimate of 100,000 visits per year for cirrhosis-related diagnosis, among which 80% corresponds to patients age > 65 years old.[3] All in all, we can state that there are multiple factors determining the success of the liver transplant patient.

Infections in liver Transplant. Infections have an important impact in the liver transplant population. Most infections in liver transplant patients occur within the first month after transplantation and can be attributed to nosocomial infections, donor-derived, or perioperative complications.[4,5] The most common type of infection post-transplantation is bacterial, representing a major complication ranging from 20-80% in patients with cirrhosis.[6] Immunological dysfunctional mechanisms against bacterial, viral or fungal infection may lead to sepsis and higher mortality in liver cirrhosis.[6] The prevalence of infection in hospitalized cirrhotic patients is of 32% to 34%, which is about 4-5 folds higher than in the general hospitalized population.[7] Hence, research has found that the most common infections in patients with liver cirrhosis are spontaneous bacterial peritonitis (SBP) (7-31%), urinary tract infections (11%) and bacteremia (12%). Indeed, bacterial infections are the cause of death in about 30% to 50% of the patients with cirrhosis, which makes a large portion of the liver transplant population.[7]

It is worth noticing that sometimes the cause of bacteremia in liver transplant patients is Vancomycin-resistance *Enterococcus faecium* from an intra-abdominal source.[1] In which many liver cirrhosis patients develop urinary tract infections mostly caused by Gram negative bacilli and staphylococci[8] because of the use of urinary indwelling catheters. To illustrate, organisms responsible for major causes of infection in cirrhosis are Gram-negative bacteria, e.g., *Escherichia coli, Klebsiella* spp and *Enterobacter* spp*,* whereas for Gram-positive bacteria it only includes 20% of the organisms, especially enterococci and *Staphylococcus aureus,* and 3% of anaerobes.[9] Moreover, gram-positive bacterial including enterococci infections are mostly related to receiving quinolones prophylaxis and invasive procedures.[5]

Antibiotic prophylaxis in liver transplant. Antibiotic prophylaxis regimen is given to liver transplant patients to decrease the incidence of pre, peri and post-transplantation infections and increase the rate of success in the post-transplant outcome. In a meta-analysis done by

Bernard et al. (1999), antibiotic prophylaxis significantly increased the percentage of patients free of infections by 32% in liver cirrhotic patients with gastrointestinal bleeding. The same study showed that short-term antibiotic prophylaxis significantly increases the short-term survival rate in patients with cirrhosis.[10] Then, the most commonly used antibiotic regimen in liver transplant are broad spectrum betalactams (25% to 75%).[6] As an example, some of the emerging pathogens associated with the use of broad-spectrum antibiotics for prophylaxis are *Clostridioides difficile* and *Streptococcus* species.[7] It is well known by clinicians and researchers that the use of broad-spectrum antibiotics promotes colonization of antibiotic resistant bacteria.[11–13] With this in mind, we can state that even though antibiotic prophylaxis may increase the risk for antibiotic-resistant bacteria colonization, in certain situations it also increases survival rate and the percentage of liver patients free of infections.

Enterococcus. Enterococci species are symbiotic commensals that commonly reside in the human urinary tract and the gut. This species, however, have the ability to colonize their habitat in the human host. To clarify, when referring to the term "colonization" as a clinical concept, the definition alludes to the presence of multiplying bacteria in the human host environment (for e.g., gut, lungs, etc.) in which the sum of all actions from the colonizing species may or may not trigger an immunological response[14] to elucidate clinical significance. Now, the term "colonization" as a concept in biology is assign to the occupation of a habitat by a single population of species in an ecological niche.[15] In biology, therefore, the term colonization is use to indicate a single population of species in their habitat without considering the species relationship (symbiosis) with the host or the effects they may have in the host homeostasis. Thereupon, both concepts could be applied when we are discussing VRE colonization in the human gut. For instance, when discussing the effects of VRE colonization and their role in the gut microbiota, emphasis on biological colonization is place as the gut microbiota itself is an ecological niche. Under the clinical scope, however, VRE colonization may be associated with bacterial infection and disease in the human host.

Vancomycin-resistant Enterococci is an important resistant bacterium in liver transplant. A frequently prevalent antibiotic resistance bacteria in Canada is Vancomycin-resistant Enterococci (VRE). VRE has the ability to colonize the host and it is oftentimes seen in patients with chronic liver diseases compared to colonization with other multi-drug resistant bacteria (MDR).[16] Importantly, there is an increasing risk of VRE colonization after liver

transplantation. In a metanalysis conducted by Ziakas, et al. (2014), the rates for pre and post-liver transplant VRE colonization are 11.9% and 16% respectively.[17] Overall, progression from VRE colonization to infection is uncommon, but it is associated with high mortality rate.[2,18] As a result, VRE infection increases hospital length of stay, odds of intensive care unit (ICU) admissions, discharge to long-term care facility, the need for major surgical procedures and health care costs.[19] It should be high-lighted that Intensive Care Units (ICUs) are known for being reservoirs for VRE (via rectal swab, 9.7 to 51.9%) and other antibiotic resistant bacteria.[20,21] What is more, VRE colonization is associated with worse survival of liver transplant patients[20], independent of the development of a clinically-significant VRE infection.

VRE colonization in the gut. VRE colonization could lead to dominance of VRE species in the gut microbiota ecosystem and, therefore, be an indicator of microbiota dysregulation. The dysregulation of the microbiota is seen once there is no diversity in the bacterial and overall gut species ecosystem. As mentioned, because of the antibiotic use during the liver transplant intervention, a disruption of a healthy gut microbiota ecosystem can occur. This goes without mentioning the already dramatic microbiota changes in patients with chronic liver disease, regardless of the antibiotic use.[22] The most commonly use antibiotics are, cephalosporins and vancomycin[23] which may allow the enterococci commensal bacteria to proliferate and colonize.[24] For example, microbiota dysregulation has been found in patients with hematological malignancies in whom Enterococcaceae almost replaces most of the healthy diverse gut microbiota when patients are colonized with VRE.[25] Other factors which may contribute to a risk in VRE colonization in post-liver transplant recipients are: poor nutrition, ischemia reperfusion injury, extended hospitalizations, immunosuppressive therapies and bacterial translocation.[26]

The gut microbiota in transplantation. The microbiota has an impact in the immunity of the host as the microorganisms belonging to the microbiome (especially bacteria) interact with the host immunological system. For this reason, it is of clinical relevance to understand how changes in the microbiota in the transplant population may have an impact in the transplant patient outcome. Current literature suggests that patients with chronic diseases present a lack of microbiota diversity.[27] Differences in the microbiota diversity across chronic diseases may advise a shift in the microbiota composition to be distinctive of each chronic disease. Liver transplant patient presenting a lack of microbiota diversity are predispose to immunological alterations, and a high risk for infections that could cause an increase allograft rejection in the

liver transplant.[26] The microbiota of the liver transplant patient is disrupted due to pathophysiological changes attributed to specific disturbances in the liver patient immunology, metabolism and due to the use of antibiotic therapy, surgery exposure and risks for infections.[28]

All in all, the present work will allow us to understand the importance that Vancomycin-resistant Enterococci bacteria colonization play in the liver transplant population and microbiota liver transplant dysbiosis leading us to find ways to improve liver transplant patient health outcomes.

1.2 OBJECTIVES OF THE PRESENT WORK

- To estimate the prevalence of VRE colonization in patients receiving a liver transplant.
- To understand the association of VRE colonization with the liver transplant health outcomes.
- To connect current knowledge of the gut microbiota with the liver transplant population health outcomes.
- To propose possible theoretical explanations of the immunological roles and mechanism by which the gut microbiota impacts transplant patient success and health outcome.

1.3 HYPOTHESIS

Vancomycin-resistant Enterococci (VRE) colonization is associated with massive dominance of VRE in stool and, hence, loss of microbiota diversity in the gut. We hypothesize that VRE colonized patients may have worse clinical outcome than those non-colonized because of disruption of the microbiome-immune and liver axis. Liver transplant patients with VRE colonization present more complications, worse clinical outcomes and higher mortality compared to non-colonized VRE transplant patients.

1.4 SIGNIFICANCE

VRE colonization can be a surrogate marker of liver transplant prognosis. For this reason, our results might set the stage for future studies in pre-clinical and clinical models in chronic liver diseases and liver transplantation. Our contribution may provide the evidence to support interventional studies targeting how to improve the gut microbiota diversity pre-transplantation, thus, providing a better understanding into the pathogenesis of the immune-liver-axis and to dig

deep into the role of microbiota in liver transplantation. By understanding how the microbiota dysregulation affects the liver transplant, because of VRE colonization, we are able to link the physiological and immunological mechanisms involved in the liver transplant outcomes.

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CHAPTER 2

BACKGROUND

2.1. VANCOMYCIN-RESISTANCE ENTEROCOCCI[1]

Enterococci are commensal bacteria that commonly reside in the gut and urinary tract of the human host. The Vancomycin-resistant Enterococci bacteria belongs to the Enterococcaceae Family under the *Enterococcu*s genus.[2] The Enterococci are a type of Gram-positive cocci bacteria for which the most commonly species found in humans as commensal organisms are the *Enterococcus faecalis* (90-95%) and *faecium* (5-10%).[2] Broadly, enterococci are harmless commensals but in certain circumstances, enterococci species (particularly *Enterococcus faecalis* and *Enterococcus faecium*) can cause infections. For instance, exposure to systemic antibiotics may lead to colonization with resistant strains of Enterococcus species, being vancomycin-resistant *Enterococcus faecium* (VRE) the most common and clinically relevant colonization species. VRE most common phenotypes are VanA (resistance to vancomycin and teicoplanin), and VanB (resistance to vancomycin alone).[3] While the most common VRE species isolated in the gut are *Enterococcus faecalis* and *Enterococcus faecium*[4], the last species is by far the most clinically relevant due to additional intrinsic mechanisms of resistant that differs from that present in *E. faecalis*. In addition, the mechanisms through which the enterococcus develop resistance is through alterations in the peptidoglycan synthesis pathway.[5] That is, there is a loss of hydrogen-bonding interaction in the peptidoglycan synthesis due to variations in D-alanyl-D-lactate, and this variations causes a six-fold loss of affinity between vancomycin and the peptide wall.[6]

VRE infection increases hospital length of stay, odds of intensive care unit (ICU) admission, discharge to long term care facility, the need for major surgical procedures, and healthcare costs.[7] Even though Enterococci can be found in the environment, VRE colonization often occurs in the hospital setting. For this reason, VRE is one of the most important multidrug resistant bacteria in the hospital admitted patients. VRE has spread worldwide and has become an increasing problem in healthcare. As a result, colonization with VRE may predispose the host to invasive infections with these strains, which are commonly initiated due to bacterial translocation from the gastrointestinal tract to distal organs, thus, leading to life-threatening situations.

Epidemiology of VRE Colonization. The prevalence of VRE colonization in the US and Canada is increasing. As a result, the number of hospitalized patients due to VRE infection doubled between 2003 and 2006 in the US.[8] It is important to recognize that enterococci reside as commensals in the jejunum and ileum with other high-density species.[9] The most common enterococci commensal species isolated in the gut are *Enterococcus faecalis* and *E. faecium*, constituting 80% and 10-20% of enterococci, respectively.[4,10]Also, VRE prevalence varies according to geographical region.[11,12] For instance, in European countries, population of the Mediterranean and UK will present significantly higher prevalence rates of VRE colonization compared to others varying from 0 to 1.2%[13,14], this happens because of VRE associations to diet and nutrition. Colonization with VRE in North America is mostly diagnosed in patients admitted to the ICU[15] since VRE colonization surveillance through rectal swab and culture is mostly done in patients admitted to the ICU. For this reason, the prevalence of VRE is higher in ICU patients. As mentioned, diet and nutrition influence the presence and isolation of VRE in individuals from different geographical regions. For example, consumption of animal-derived food products in Europe is a usual source of VRE colonization suggesting that finding VRE in the gut of healthy individuals is common.[15] Since the initial recognition of VRE from patients in the United Kingdom and France, the presence of VRE bacteria have been found all over Europe including countries such as, Belgium, Denmark, Germany, Italy, The Netherlands, Spain and Sweden.[16]

Risk of VRE infection in VRE colonized Patients. The most important risk factor for VRE colonization and infections is exposure to broad spectrum antibiotics. As mentioned, VRE colonization occurs mostly in the gut, therefore, bacterial translocation is possible leading to systemic VRE infection in patients[17], including nosocomial surgical site, genitourinary, intra-abdominal and bloodstream infections.[18] In spite of the limited number of antibiotics for the management of VRE infection, the adverse effects associated with these treatments are high. Managing VRE infections is complicated and associated with high morbidity and mortality.

Bloodstream infection (BSI) is the most common and typical form of VRE infection[19,20], mainly affecting the adult frail population. Among colonized patients, VRE BSI rates range from 0% to 45% depending on the population of study.[21–23] Independent risk factors for VRE BSI are long-term care facility, infection of an additional body site and exposure to vancomycin.[23] Importantly, prevalence of VRE infection are especially highest in solid organ transplant (SOT), including liver transplant (LTx), hematologic-oncologic and critically-ill patients.[21] In spite of

immunosuppressed patients presenting the less cases of VRE infection[21], their susceptibility for developing VRE infection may be associated with the dosage of immunosuppression therapy. For this reason, VRE is a less virulent organism in comparison to other gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* that might overpopulate regardless of the degree in immunosuppression, however, it is still one of the most prevalent colonizing bacteria in the liver transplant population and may predispose to BSI.[22]

Association of the VRE colonization in the gut microbiota. Disruption of the microbiota diversity because of VRE colonization could result in commensal bacteria colonizing the gastrointestinal tract. Enterococci are commensals that could colonized the intestinal tract if there is a disruption of normal microflora due to exposure to antibiotic treatment, especially cephalosporins and vancomycin.[11,24] For this reason, the use of antibiotic treatment itself may lead to an alteration in the mucosal barrier structure, henceforth, debilitating the defensive innate immune system and its mechanistic pathways.[25] Microbiota dysbiosis in the gut has been found to be involved in various chronic diseases when the symbiotic relationship with the host is disrupted. That is, the host relies on the microbiota for important metabolic and immunological processes. If the microbiota is disrupted because of antibiotics use, the commensal bacteria ecosystem draws down enabling the growth of VRE and other antibiotic-resistant pathogens.[26,27] The persistence of VRE in the gut after the discontinuation of antibiotic treatment may suggest that the consequences of the dysbiosis by cause of antibiotic therapy could have a long-lasting effect.[28] Furthermore, the presence of other bacteria in the gut could act as VRE colonization antagonists. For example, in a study done in mice models, the eradication of VRE from the gut microbiota occurred when the intestinal flora was recolonized with anaerobic bacteria from the *Barnesiella* genus.[29] Also, the presence of cephalosporinase-producing *Bacteroides thetaiotaomicron* showed to prevent VRE from expand and colonize in the gut of mice models.[30] For this reason, VRE colonization may be consider as a surrogate marker of health contributing to worse outcomes, complications, increase in the length of hospital stay, exposure to broad antibiotic regimen and the need for invasive procedures.[31]

Immune system responds to the growth of pathogenic bacteria. Some innate proteins of the immune system play a role in preventing intestinal colonization. As illustrated in Figure 3 below, in a study done by Brandl and colleagues, they showed that the use of antibiotic treatment downregulates the intestinal expression of the innate immune effector RegIIIϒ in mice.[32] As

previously understood the immune effector RegIIIϒ plays an important role in the killing of gram-positive bacteria such as VRE.[25] In another study, the role of commensal bacteria was found to be important in preventing VRE gut colonization.[33] For example, commensal bacteria may proceed to activate toll-like receptors 4 and 5 through binding of lipopolysaccharide and flagellin receptors, respectively. In such instances, TLR 4 and TLR 5 act upregulating the production of RegIIIϒ.[25,32,34] Finally, other research[32,34] have confirmed these findings studying the indirect activation of the innate immune system through VRE expansion. In spite of these findings, clinical information on the role of RegIIIα (the human ortholog of RegIIIϒ) involving VRE colonization and infection is non-existent.[35]

The establishment of VRE colonization is a risk for life-threatening VRE infection. For this reason, translocation of bacteria via bloodstream as a result of VRE colonization has been correlated with all types of surgical procedures in animal models.[36] Another example of translocation that occurs via lymphatic system has been seen in patients with cirrhosis suggesting that the growth of VRE is significant in the mesenteric lymph nodes.[36] Figure 2 illustrates the growth of VRE in the mesenteric lymph nodes as the original infection site, possibly resulting in VRE to cause a systemic infection via bloodstream. (See illustration, Page 5).

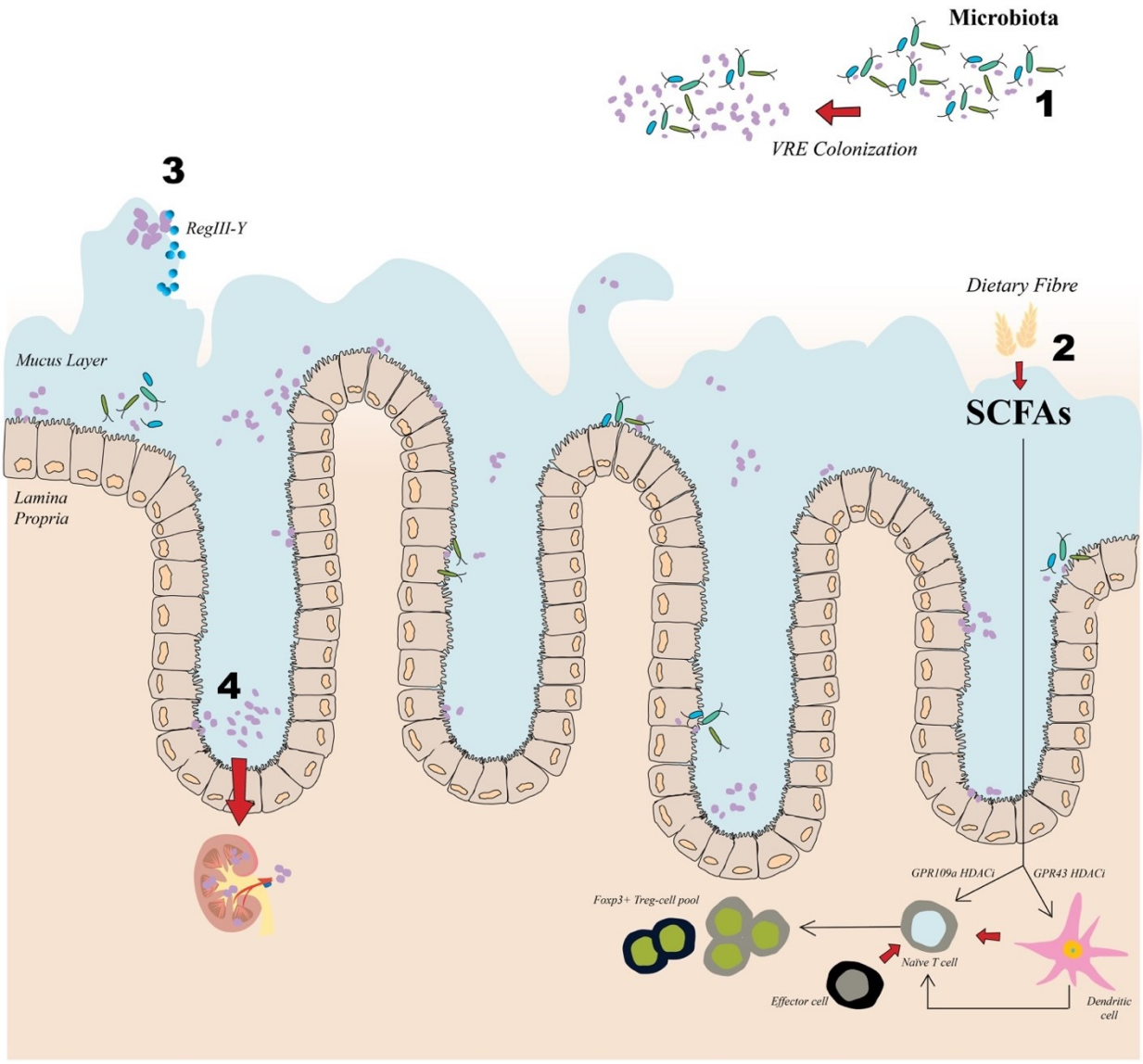

Figure 2. Pathophysiology mechanisms of VRE colonization in liver transplant patients. **1.** *Bacteroidetes*, *Firmicutes* and *Actinobacteria* are three important members of the microbiota species that induce and modulate the innate and adaptive immune responses suppressing pathogenic bacteria and VRE overgrowth.[26] In such cases, bacterial interactions, clearance and cooperation may be the result of intricate processes.[37] Importantly, the lack of microbiota diversity (*dysbiosis*) often allows for VRE to dominate and colonize the intestinal gut. **2.** The human microbiota has a direct influence in a number of metabolites, most importantly, the short-chain fatty acids (SCFAs). SCFAs are generated by commensal microbiota members as a result of anaerobic fermentation in the gut from dietary fibre, especially by Clostridia species. The most studied SCFAs are acetate, propionate and butyrate, these are chemical compounds made of carboxylic acid moiety and a small hydrocarbon chain. SCFAs contribute to the induction of pTreg cells. SCFAs act as inhibitors of histone deacetylase (HDACi) while entering dendritic cells, in order to suppress the expression of pro-inflammatory cytokines.[38] SCFAs also act directly on naive T cells through GPR43 or the upregulation of Foxp3 expression over HDAC inhibition.[38] **3.** RegIIIγ is an innate immune effector or defensin playing a role in killing of gram-positive bacteria, including VRE.[37] To begin, RegIIIγ is expressed in intestinal epithelial cells and Paneth cells of the small intestine.[39] The loss of commensal gram-negative anaerobic bacteria leads to a reduce binding of lipopolysaccharide and flagellin to TLR4 and TLR5, respectively, and enhancing the downregulation of RegIIIγ, which produces VRE overgrowth. For example, in a study done in mice, RegIIIγ expression was found to be dependent on interleukin 22 expression as well as TLR5 expression.[39] **4.** Data from animal models of VRE-colonized rats demonstrated that bacterial translocation of VRE occurs in blood and lymphoid tissue.[36] For example, VRE was found to migrated from lymphatics to mesenteric lymph nodes in ischemia-reperfusion injury rat models.[36] The ability of VRE to migrate through lymph nodes in animal models, may suggest a reservoir for potential invasive infections in humans, therefore, resistance to decolonization. In addition, the presence of VRE in lymph nodes may perpetuate inflammation and/or T-cell activation. Moreover, recolonization with VRE is common and may be attributed to factors in the host that contribute to persistent dysbiosis. Overall, alterations in the function of the intestinal barrier due to multiple insults is common in the post- liver transplantation, thus, VRE translocation to the lymph nodes may be a suggestive sign of impending invasive infection contributing to these alterations in the intestinal barrier.

Most studies in the transplant patient population have determined an association between the microbiota profile phenotype and the bacterial composition determined through clinical endpoints and worse outcomes, including endpoints such as acute rejection and infections.[27] The sample size of these studies in renal transplantation, however, is not enough to considered the changes in microbiota characteristics as predictive biomarkers for pre- and post- transplantation outcomes.[27] Microbiota researchers have established that understanding the functional characteristics of the microbiota is the key to link the microbiota role to complications and health outcomes. That is, knowing the phenotype profile of the microbiome is not enough to determine the impact of the microbiota in chronic diseases and solid organ transplantation health outcomes. These findings in solid organ and hematopoietic stem cell transplants have significantly concluded that the microbiota composition is correlated to complications, such as, acute and chronic rejection, diarrhea, graft-vs-host disease and infections.[40,41]

The gut microbiota of liver transplant patients presents a lack of diversity associated with immunosuppression therapy, antibiotic exposure, surgery, invasive procedures, metabolic alterations and infection.[42] As an example, *Bifidobacterium dentium* has been found to appear as an opportunistic pathogen in liver transplant patients contributing to the loss of microbial diversity, hence, increasing the risk of infection linked to extended used of antibiotics/antimicrobial therapies.[43]Also, liver patients with chronic disease, especially cirrhosis that have been exposed to antimicrobial drugs, present a lack of gut microbiota diversity that is often resolved after undergoing liver transplantation.[44] What is more, liver transplant recipients with cirrhosis are predominant in *Enterobacteriaceae* and *Enterococcus spp.* when compared to healthy controls.[44] Nevertheless, in liver transplantation there is a high prevalence of *Enterococci,* and as a result, acquisition of VRE is more likely to occur in the liver transplant population.

Association of VRE with transplantation. Transplant patients undergo antibiotic exposure placing them at risk for VRE colonization. For instance, vancomycin, fluoroquinolones and third and fourth generation cephalosporins are specific antibiotics correlated to predisposition for VRE colonization.[45,46] Furthermore, additional risk factors such as prolonged hospitalizations, ICU admission and in the ICU setting, patients with diabetes mellitus, chronic heart failure, chronic obstructive pulmonary disease and chronic renal failure are placed at higher risk for VRE colonization.[47–49] Since early post-transplantation, liver transplant recipients present

a lack of microbiota diversity, that in combination with other risk factors including malnourishment, ischemia-reperfusion injury, antibiotic exposure, and immunosuppressive therapies leads to VRE colonization.[50]

There is an important bidirectional relationship between immunity and gut dysbiosis. As a result, immune dysregulation and microbiota dysbiosis are present simultaneously placing transplant recipients at a higher risk for infection events and worse outcomes in liver transplant.[50] For these reasons, it is important to consider incorporating the proper use of perioperative antimicrobial prophylaxis in the liver transplant as it could serve to target VRE colonization post-transplant and prevent VRE infection.

Outcomes of VRE colonization in pre- and post-transplant patients. Studies have shown that VRE colonization may be associated with increased mortality in the transplant setting.[51] For example, in one study VRE colonization carried a mortality rate of 7% in liver transplant candidates and recipients.[33]Another study showed a 12% mortality of patients colonized with VRE, in spite of death not being attributed to VRE infection.[48] Certainly, studies have suggested that VRE infection, particularly VRE BSI, is an independent risk factor for death.[52] The gut microbiota, however, does play a role in the increase of VRE BSI when the presence of VRE bacteria in the gut microbiome is over 30%.[53]

A study done by Russell and colleagues reported that there is a 60% 1-year mortality in VRE colonized liver transplant candidates and recipients.[54] VRE colonization during the post-operative period in non-colonized liver transplant recipients significantly increases the risk of VRE infection and subsequent mortality.[55]Also, current evidence suggests that there is greater mortality at 90 days post-LTx in patients who acquired VRE.[56] To illustrate these findings, a study in liver transplant recipients showed that VRE colonization led to significantly longer preoperative hospital stay and higher Model for End-Stage Liver Disease (MELD) scores when compared to non-colonized patients.[55] Henceforth, emphasizing the importance in considering acquisition of VRE colonization post-liver transplant and its role in the overall post-transplant complications such as, long-term hospital care and stay post-surgical course, including long stays at the ICU is needed. This data may suggest that mortality rate associated with VRE infection is high and relevant in the transplant patient population.

Patients undergoing transplantation receive a degree of immunosuppressive therapy that may have direct association with the risk and development of enterococcal invasive disease. Thus,

patients with end-stage liver disease and liver transplant recipients are at particularly high risk for life-threatening infections. Addressing VRE could be challenging as there are not many effective antimicrobial drugs resources that would out weight the benefits of therapy over the adverse effects of drug administration in transplant. For instance, in a historical cohort study, cases of VRE bacteremia were matched to patients with vancomycin-sensitive enterococci (VSE) bacteremia mortality was attributed to VRE bacteremia in 37% (95% CI: 10%-64%) of the study population.[57] In addition, other studies have demonstrated vancomycin-resistant to be an independent risk factor for death in patients with enterococcal BSI.[57–60]

As previously mentioned, the exposure that solid organ transplant recipients have to antimicrobials and immunosuppressants invariably alters the microbiota ecosystem and community that may lead to drastic effects in outcomes. This occurs as a result of the complex interactions between the microbiota and the immune system of the host.[61] The microbiota-liver axis is of special relevance in the liver transplant patient population due to the metabolic, anatomic and physiologic interdependence interactions between the liver and the gut.[62] To summarize the importance of VRE colonization in liver transplant candidates and recipients: VRE may act as a marker of microbiota dysbiosis leading to changes in the functional impact of the gut microbiota, and consequently affecting systemic immunity. VRE colonization could also be a predisposing factor for VRE infections associated with morbidity and mortality.

2.2 RELATIONSHIP BETWEEN VRE COLONIZATION AND THE MICROBIOTA

The presence of VRE in the gut may result as a consequence of the microbiota dysregulation during chronic liver disease. Furthermore, the opposite could also be true as VRE colonization could lead to microbiota dysregulation in the gut in patients with chronic liver disease, thus, contributing to the progression of the liver disease. A diverse microbiome ecosystem in the gut enhances resistance to pathogenic colonization such as VRE. For example, the lack of microbiota diversity alters the production of the antimicrobial lectin (RegIIIγ) which targets Gram positive pathogens including VRE, as healthy microbiota commensals promote RegIIIγ production.[25]Also, commensal bacteria may kill and control for the expansion of other bacteria through production of molecules and peptides such as bacteriocins and microcins.[63]As an example, *Escherichia coli* Nissle 1917 probiotic produces microcins limiting the expansion of other Gram negative bacteria such as enterobacteria.[63] The presence of Enterobacteriaceae is

well known for its contribution to gut inflammation and its presence is considered to be a hallmark of dysbiosis.[64] Current research has shown that an increase abundance of other taxa in fecal samples belonging to Proteobacteria phylum, specifically the Enterobacteriaceae family predicted with accuracy if patients were colonized or not with VRE pre-Fecal Microbiota Transplant (FMT).[65] The study showed a relative abundance of 30% of Enterobacteriaceae in the VRE colonized patients pre-FMT suggesting that Enterobacteriaceae in the stool could be a risk factor for VRE pathogenic colonization.[65] In a study done in the allogeneic hematopoietic cell transplant (allo-HCT) population, enterococcus expansion was found to be associated with graft-versus-host diseases and mortality.[41] Also, smaller single-center analyses have demonstrated that VRE bacteremia and colonization are associated with worse health outcomes post allo-HCT.[66–68] For this reason, VRE colonization in liver transplant patients may also lead to worse health outcomes after transplantation since the presence of VRE colonization could also be a hallmark of dysbiosis, disrupting the production of important bacterial metabolic products, thus, contributing to the worsening of secondary endpoints such as clinically significant infections, allograft acute rejection and acute kidney injury in the liver transplant population.

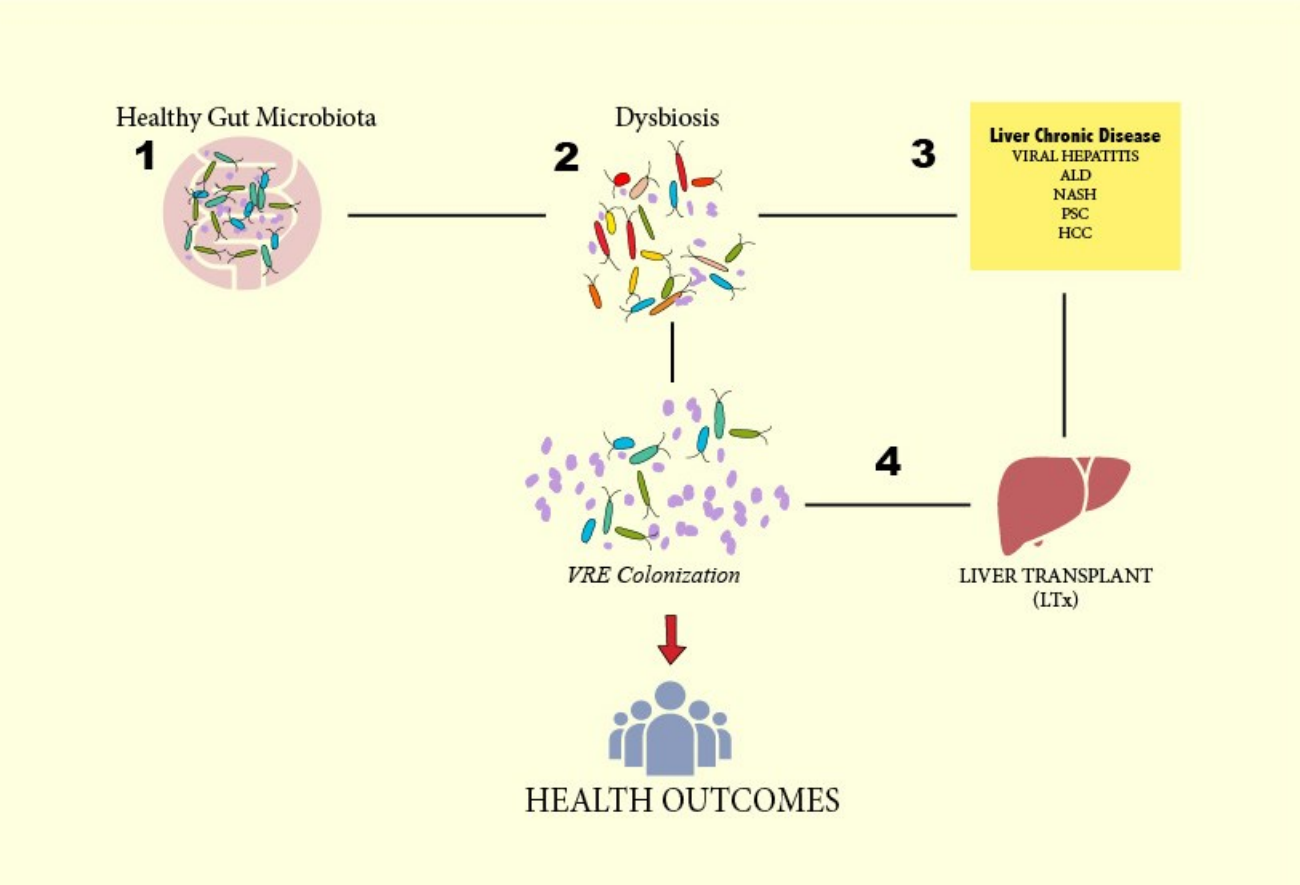

Figure 3. VRE colonization and Microbiota dysregulation. 1. Healthy gut microbiota contains a diverse ecosystem and protects the gut from pathogenic bacteria to colonized. 2. Lack of microbiota diversity *"dysbiosis"* occurs as a result of gut microbiota ecosystem disruption. The disruption may be a consequence of pathology, microbial ecosystem interaction and competition. Dysbiosis may lead to alteration in pathogenic colonization resistance. For e.g., Reduction of RegIIIγ, microcins and bacteriocins[25,63,64]and may also promote VRE colonization.[65]3. Dysbiosis in chronic liver diseases may be resolved after liver transplantation restoring homeostasis and metabolism. 4. However, since VRE colonization is abundant in the microbiota environment,[69]VRE colonization pre and/or post-transplant could be a hallmark of dysbiosis leading to worse health outcomes peri and post-transplantation as restoration of microbiota diversity is not achieved in presence of VRE colonization.

2.3 THE HUMAN MICROBIOTA

The human microbiota, also known as microbiome, is a term commonly used to describe the complex communities of microorganisms and ecological niches inhabiting multiple human tissues and body surfaces. Microorganism conforming the microbiota include, bacteria, virus, parasites, protists, yeast, Archaea and Fungi.[70] In order for the microbiota to establish a harmonious relationship with the host, the microbiome microorganisms have adapted to live in a symbiotic relationship with the host sharing a relationship of mutual benefit. These commensal organisms can be found in the gut, lungs, vaginal tract, urethra and bladder, oral cavity, skin, among other human tissues.[71]

Microbiome transmission. As a result of the non-sterile nature of most human tissues, communities of the microbiome microorganisms are found in different body surfaces and are acquired through horizontal and vertical transmission.[71,72] In fact, the microbiota composition among great ape species is phylogenetically conserved and has diverged in consistence with vertical inheritance.[71,72] In addition, bacteria are also acquired through horizontal transmission, that is to say the gut is continuously and initially seeded with bacteria establishing early microbiome traces.[71,72] For instance, some bacteria lineages, like *Lactobacillus reuteri* are relevant in the study of the microbiome as they maintain stable associations with specific vertebrates over evolutionary timescales.[73]

Human colostrum and the microbiome transmission. The human colostrum/milk is the main source of nutrition during the first year of an infant's life. The colostrum bacterial composition includes about 200 different species[74] namely, *Staphylococcus*, *Streptococcus* and *Bacteroides* genera, together with probiotic bacteria such as *Alloiococcus* spp.[75] For this reason, colostrum nutrition impacts and establishes the gut microbiome species since early life span through vertical and horizontal transmission. That is to say, from birth until 2-3 years of age. Thus, breastfeeding is accounted for the extensive presence of Bifidobacteriaceae, Clostrodiaceae, Lactobacillaceae and Lachnospiraceae species during the first year of life and the establishment of the gut microbiome.[76] Likewise, milk provided over breastfeeding has proven to impact the microbiome due to its glycobiome component.[77] For example, oligosaccharides pertinent to human milk bide to pathogenic bacteria and trigger protective mechanisms in the host, henceforth, allowing the growth of beneficial bacteria in the gut.[77]

Evolutionary history of the host-microbiome relationship. The relationship between the human host and the microbiota has its evolutionary history. For starters, the holobiont perspective could be used to illustrate the microbiome co-evolutionary history with the human host.[78–80] The term holobiont was first introduced by Lynn Margulis in 1991. Margulis proposed the term as an explanation for the endo and exosymbiosis relationship between the host and its microorganisms residents.[84] In spite of the co-evolutionary relationship and development between the host and the microbiome, the biological entity of the microbiota and the host present distinct and separate function in metabolism, anatomy and immunology.[81]

To understand the evolution of the microbiome in humans, scientists have studied the development of the gastrointestinal tract in different species. Likewise, factors such as, diet and

the mechanisms by which microbes contribute to the production and conservation of nutrients in the host have also been studied.[82] For instance, vertebrates maintain microbial populations in their gastrointestinal tract because the microbial populations provide essential benefits to the host. As an example, hindgut fermentation of most terrestrial vertebrates and foregut fermentation contribute to larger and greater diversification of the host microbiota.[82] Therefore, bacterial fermentation and microbiome diversification, has resulted in microbes producing metabolites that contribute to the host evolutionary fitness.[83]

Establishing the host microbiome. There are two fundamental factors allowing the host to select and establish their individual microbiome. The first factor is environmental (for e.g., environment, diet, history of antibiotic use, etc.) and the second factor is host genetics. Current literature presents debating evidence on the existence of a core human microbiome.[84] What is more, individuality in the gut microbiota composition relies on complex polygenetic trait shaped. Host genetics plays a role in shaping the diversity of the microbiota in mammals. As an example, around 18 host quantitative trait loci (QTL) has been identified showing and suggesting their link to the relative abundance of specific microbial taxa.[84] For this reason, different hosts have their own specific gut microbiota consisting of many host specific lineages and there are at least over 50 core taxa found in a significant sample of human subjects.[85,86]

Variation in the gut microbiome can be seen among human populations due to diet and geographical region. For example, western societies may present different microbiome profiles compared to individuals from non-industrialized societies. To illustrate, in a study done by Smits et al. on the Hazda hunter-gatherers of Tanzania, researchers found that the microbiome profile of the Hazda tribes share more similarities associated with a plant-based diet.[87] This plant-based diet microbiome is closely related to the microbiome profile of ancient co-evolutive species.[87] Members of the Hazda tribes presented a microbiome profile dependent on a seasonal cycling pattern. Thus, when compared to the microbiome of western society's individuals, the Hazda operational taxonomic units (OTU), highly deferred from each other.[87] For this reason, there is a fundamental need to understand the ecological role and functional contributions of individual bacterial species and the species co-evolution with humans since certain species seemed to be missing or underrepresented in the microbiome of industrialized populations.

While primary individual microbiota reflects the maternal hand-over ecology at birth[88,89] a series of complex and dynamic interactions between diet, life-style, disease and antibiotic use

has shaped the microbial landscape evolution across lifespan. The topographical and temporal variation in the microbial communities has influenced the composition of fecal microbiome within and between individuals. This developmental trajectory of the microbiome modulates the metabolic profile of the host influencing its disease susceptibility.[90]Although changes are temporal, the adult microbiome is dominated by species and strains which form stable and resilient population composing an ecosystem that could suffer substantial alterations due to antibiotic dosage.

The composition of the gut microbiota in response to diet are highly individualized in humans. Regardless, the association between diet and the microbiome may not seemed to highly contribute to the inter-individualization of beta diversity as diet could rapidly alter the gut microbiome inter-individual ecosystem variations.[91,92] Factors involving lifestyle may disrupt the symbiotic interactions with our microbiome leading to pathology. Fundamentally different adopted lifestyle from the diet under which the human microbiome interrelationship evolved, might have disrupted this symbiosis. Therefore, reducing or removing the evolutionary routed benefits that the microbiota organisms provide. As a result, microbiota dysregulation associated with lifestyle factors might have contributed to the rise of health complications and chronic diseases linked to the microbiome.

2.4 THE GUT MICROBIOTA

The gut microbiota includes over 1,000 bacterial species, with a large portion of Archeae and Fungi (phyla Ascomycota and Basidiomycota).[93] There are more microbes in the gut than cells in the human body. The gut microbiome encodes around 150-fold more unique genes than the human genome. For this reason, the microbiome is often consider as an adjunct organ and second genome.[94] Certainly, the microbiota constitute 90% of the total number of cells associated with our bodies; only the remaining 10% are human cells.[95] For example, one type of microorganism interacting with the microbiome is bacteriophages. The role of the bacteriophages is important because they are part of the microbiome genetic encoding. Bacteriophages provide genetic variety since they integrate into the bacterial genome.[96] The microbiome host genotype association goes in hand with co-evolution and microbiota genes that can be assigned into functional pathways or categorical groups. For this reason, modulations through host genetics could impact the abundances or presence/absence of microbial genes.[97] The microbiome

attributes are of high relevance as the understanding on how the host genome might influence the microbiome is unclear.[98] There is, however, growing evidence stating that epigenetics triggered by the microbiome is responsible for functional implications of the host homeostasis.[98]

The microbiota along the gastrointestinal tract. Bacterial species found within the gut are Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia[99,100] The main genera of the Bacteroidetes group are *Bacteroides* and *Prevotella.* Importantly, Gram-negative anaerobic rods make up about 25-50% of the human colonic microbiota and, most Gram-negative bacteria can metabolize carbohydrates,[101] peptones, and/or metabolic intermediates. Along the gastrointestinal tract, the distribution of the gut microbiome is not homogeneous. For example, the largest proportion of microbes are mostly anaerobic bacteria and they resides in the colon portion of the gut, and there are variations in diversity, genre and bacterial numbers along the gastrointestinal tract (GI).[97] The upper GI tract comprising the stomach and small intestine, present high pH and shorter transit time, for which the amount of bacteria present in this area is lower (about 10^3 to 10^4 bacteria mL^{-1} of intestinal content).[95]

In the large intestine there is a high prevalence of Bacteroides, Firmicutes, Bacteriophages of Bacterioides, Firmicutes and Actinobacteria, so as a major diversity in Ascomycota, (Candida, Saccharomyces, Penicillium, Cladiosporium, Galactomyces, Cryptococcus), among others.[102] Notably, the largest portion of microbes reside in the colon, in which, most bacteria are anaerobic[103] because of the oxygen gradient in the mucosa that provides a competitive advantage for facultative anaerobes.[104] Over 90% of healthy anaerobic colonizing bacteria belongs to the Firmicutes and Bacteroidetes phylum. The ratio among Firmicutes and Bacteroidetes differ from one individual to another depending on a variety of factors,[105]so as the stability of the microbiome along the GI tract.[106]

Most of the microbiota and undigested foods are found in the lumen, a central space of the intestinal tract surrounded by the layer of tubular intestinal mucosa. A process of substrates absorption takes place through mucosal epithelial cells and prevents the entry of the microbiota into the intestinal mucosal cells of the host.[104] To a certain extent, the host-microbe interaction occurs mostly within the mucosa. Moreover, secretion of metabolic products in the intestinal tract from the microbiota contributes to the epithelial metabolism. This is very important as microbiota metabolites are a significant source of energy, for example, short-chain fatty acids (SCFAs) like butyrate.[107] To remark, The importance of metabolic products from trans-

Kingdome microbiota to host physiology and homeostasis have been studied by different groups.[108,109]

As previously stated, most interactions between the microbiota and the host occurs through contact of the bacteria and the gut mucus layer. For example, *Akkermansia mucinophila* is one example of bacteria that plays an important role in maintenance of the mucus layer. *Akkermansia mucinophila* species can be found residing in the mucus layer feeding on mucin. Because of the role *A.mucinophila* plays in the host gut, in pathophysiological conditions this species may cause detrimental degradation of the inner tubular mucosa allowing other microorganisms to transpose from the lumen into the host cells triggering immunological responses that may lead to homeostatic disturbances.[110,111] Effects of interactions between the gut microbiota and the host, especially from the microorganisms located in the large intestine, have an impact over the human health homeostasis including, energy absorption, maintenance of mucosal layer, development and establishment of immune system.

Functional characteristics of the gut microbiome. Functional characteristics of the gut microbiome impact the host metabolism significantly. Some functional characteristics include, degradation and fermentation of indigestible nutrients into absorbable metabolites, synthesis of vitamins, out competition of pathogenic agents, elimination of toxic compounds, enhancement of the intestinal barrier, regulation and development of the immune system.[84,112–114] Functional impact that the gut microbiota has in the host is tightly intertwined with human physiology. For example, metabolic products derived from microbial fermentation (e.g., short-chain fatty acids, SCFAs) are known for playing an important role in immunomodulatory processes such as T-cell differentiation. These immunological processes could create a feedback loop as they may affect in return the gut microbiome stability and diversity. Importantly, microbial community function and structure differ from each other.

Microbial community structure is known as the numbers and types of microbes present in the microbiota ecosystem (phenotype), whereas the microbial community function is related to metabolic activities and products resulting from microbial activity.[115] As a result, the association between microbial communities' structure and function are strong.

Functional implications, however, may be of major relevance and importance in measuring the microbiome stability and healthiness in relationship to the host. Therefore, distribution of different functional genes emerging from the microbiome may carried out key functions

suggesting that functionality is more important than the specific microbe's species needed to be present to carry those functions out.[116] Omics are fundamental in studying the functional role of bacteria in the host. As an illustration, metatranscriptomic, metaproteomic, and metabolomic analyses would allow to assess functionality of the gut microbiome.[117–121] Study of functional omics would present a more accurately portray health and disease states[120,122] For example, fermented milk products have shown to be responsible for changes in gene expression, which, in this case is linked to dietary interventions[123]and the oral intake of medication.[121] Metagenomics data and metagenomics functional profiles present less variability in comparison to taxonomic profiles.[116] Since the specific taxonomic species cannot be isolated and assigned to particular functional roles, it is crucial to understand the profile of the healthy core microbiome and its individual metabolic pathways in order to assigned specific functional activity. For this reason, further studies to assess the functional aspects and roles of the microbiome in the host will continue to increase in the coming years as we move from simple listing and cataloguing of the microbial taxa and their genes into understanding and modeling the entire microbial community. Consequently, providing better knowledge on the potential and functionality of the human microbiota.

2.5 THE IMMUNE-MICROBIOME AXIS

The gut microbiota has the ability to induce changes in the immunity of the host because the microbiota microorganisms maintain direct and indirect interactions with and within the host due to symbiosis. As mentioned in previous sections, the microbiota has evolved in a way that their symbiotic relationship with the host and the host's immune system is not disrupted. For this reason, the microbiota and immune system induce protective responses against pathogenic microorganism and maintain regulatory pathways that are involved in the tolerance against innocuous antigens.[124]

Early interactions between the microbiota and the host are known to set the mucosal tone and systemic immune system long term. The pre-weaning, which occurs right after birth, is the stage in which nutrition is only acquired through the sucking of milk in mammals. For this reason, during the pre-weaning interval, exposure and encounter with microbial antigens are important for the host to develop commensal and symbiotic relationship with the microbiota. Once the critical pre-weaning phase is reached, it leads to a drop in the levels of epidermal

growth factor (EGF) in breast milk.[125]As a result, high levels of EGF block the formation of goblet-cell associated antigen passages (GAPs).[125] Then, GAPs allow antigens from the lumen to enter the lamina propria. Consequently, the Treg cells or regulatory T cells, which are a specialized subpopulation of T cells that maintain homeostasis and self-tolerance through suppression of the immune response,[126] start to develop during this period of exposure. Eventually, GAPs become blocked and the antigen exposure ceases. Henceforth, setting the mucosal tone of the host immune system long-term due to first microbiota exposure.[125]All in all, failure of exposure during this period may result in a lack of development of Treg cells and a more inflammatory reaction to gut microbes later in life.[125]

Microbes, metabolites, IgA immune cells and cytokines can be found in breast milk and colostrum.[74,75] For this reason, breastfeeding and maternal milk define early responses from the host against commensal bacteria and establishes the microbiome within the first couple of years after birth.[74,75] The immunological impact of the microbiome in the host and the maternal IgA starts by restricting immune activation and attachment of the microbes providing the expansion of the microbiota constitution through addition of *Bifidobacterium.*[127] As a result, restriction of immune activation and expansion of the microbiota occurs through the binding of nutritional and microbial antigens, so as the presence of the mother's milk oligosaccharides.[127,128]

Relative immunological immaturity could also explain the molding disposition of the neonate immune system at birth, therefore, making it more accepting in the establishment of the microbiota. For example, a type of regulatory response that might ensure the establishment and stability of the microbiome during development occurs due to the ongoing increase in release and activation of inflammatory cytokine production, T and B cells.[129,130] Importantly, during this period of microbiota establishment, blunted immune responses are seen in an immature immune system making them highly susceptible to infections.[129]

The ongoing dialogue between the microbiota and the neonate. There is an ongoing dialogue between the microbiota and the infant host. The primary dialogue starts with the recognition of conserved microbial associated molecular patterns (MAMPs).[130] That is, MAMPs are signals that could help the immune system to recognize and discriminate microorganisms through Toll like receptors (TLRs), hence, eliciting the appropriate immune response. For this reason, part of the innate immune system of the neonate signal integration occurs through TLRs ligands and the microbiota. Likewise, commensal bacteria also contribute to the development of

intestinal tertiary lymphoid structures (e.g., isolated lymphoid follicle or crytopatches) through commensals exposure.[131,132] Other ways in which commensals might contribute to the enhancement of the intestinal barrier are through promoting maturation and angiogenesis of epithelial cells.[133]All in all, these early interactions between the microbiota and the neonates might play an important role in the immune responses of the host during lifespan.

Interactions between the host and the microbiota maintain gut homeostasis and immune tolerance against pathogenic agents. Microbial diversity can be found in the intestinal lumen. For instance, microbial diversity includes important anti-inflammatory species that through their interaction with the gut mucus layer induce immunological responses in the host. During the host homeostatic state, the thick mucus lining of the gut epithelial cells in the intestinal lumen acts as a physical barrier excluding most micro-organisms through compartmentalizing of commensal and symbiotic bacteria within the lumen.[134] Consequently, a process called "neutralization" occurs in the gut mucus because the intestinal epithelial cells (IECs) produce antimicrobial peptides and the intestinal B-cells release secretory IgAs.[135] Furthermore, IgA is secreted and recognition of microbe-specific epitopes occurs through binding to facilitate removal[135] and secretion of antimicrobial peptides directly neutralizing microorganisms. Another mechanism that promotes compartmentalization in response to several cues is the production of IL-22 through the intestinal immune system. This compartmentalizing response also occurs in the IECs.[135] In brief, bacterial and host interactions are limited by the physical and biochemical barriers of the intestinal wall that exists between them.

Immunological signaling and the microbiota. Several bacteria could impact the results of systemic outcomes in the host even at distal sites because they transmit signals affecting the immune and adaptive systems. On the one hand, the microbiome trigger signal responses of TLRs through binding with the IECs eliciting an immune signaling in a direct manner.[136] On another hand, a more indirect interaction occurs when the bacterial metabolic products bind with distal organ receptors during bacterial metabolites translocation. Additionally, an important contributor of the microbiota-immune axis is lipopolysaccharide (LPS). LPS is an endotoxin found in the outer membrane of Gram-negative bacteria towards which the microbiota ligands present early response, making the gut epithelial cells less responsive to TLRs stimulation.[137] To remark, these indirect responses have found to be increased during pregnancy and lactation in mouse models.[138]

In homeostatic situation, the microbiome will elicit anti-inflammatory responses producing transforming growth factors (TGF)-B and interleukin 10 (IL-10) from the IECs and mononuclear cells.[139]For example, the capsular polysaccharide A of *Bacteroides fragilis* has been found to stimulate production of the anti-inflammatory cytokine IL-10 through Treg cells.[140] Immune modulation occurs because of the prostate specific antigen (PSA) process when *B. fragilis* is taken up by dendritic cells in the lamina propria, thus, continue to be processed and presented to naïve CD4+ T cells.[141] PSA can also alter CD4+Th1 –Th2 balance and shift the balance of effector T cell subsets in the spleen.[141] Most of the immune-microbiome axis interaction is reliant on the metabolic products of the microbiota.

During a microbiota dysbiosis state, the immune response elicited by the IECs would stimulate the mononuclear cells and lymphocytes through liberation of pro-inflammatory cytokines.[142] These signals and molecules that may play a role in the anti-inflammatory response are B-cell activating factor (BAFF), short-chain fatty acids (SCFAs) and tumor necrosis factor (TNF).[142] To illustrate these complex interactions of the microbiota with the host immune system, an experimental model of autoimmune encephalomyelitis (EAE) demonstrated that Tregs produce IL-10 in response to the metabolites SCFAs and PSA protecting against inflammation. These metabolites are microbial derivate especially from *B. fragilis and Clostridium*. What is more, activation of non-canonical autophagy through protein ATG16L1 and the receptor Nod2, induce Treg cells suppressing mucosal inflammation.[143] Similarly, *Clostridium ramosum* is a potent inducer of colonic Treg cells.[144]

Toll-like receptors (TLR). Toll-like receptors have a specialized response in IECs making them of high importance in modulating the immune response together with the microbiome. IECs are polarized type of cells that play a role in the distinct trafficking and regulatory mechanisms between the IECs and TLR precisely in relation to location and individual TLR signaling.[145] Two of the most relevant TLR modulating immune responses and signaling with the microbiome are TLR4 and TLR2.[145]

TLR4 are involved in defense against pathogens. TLR4 is downregulated in IECs in homeostatic conditions but increased their activity and expression during inflammation and intestinal injury.[145] If TLR4 signaling is disrupted or absent this could lead to bacterial translocation, systemic disease and severe local damage involving the mucosa. TLR2 can produce both, anti- and pro inflammatory immune responses.[145] For this reason, TLR2

interactions with multiple receptors makes it a good modulator and TLR2 also produce direct activation on CD4 T cells promoting the differentiation towards Th1 or Th17 cells.[145]Likewise, TLR2/6 helps in the tolerance of dendritic cells,[145] since when activated directly on IECs, TLR2 promotes barrier function through tight junction effects.[145]

Bacterial translocation could result from chronic intestinal inflammation**.** As an example, in chronic intestinal inflammation activation of events such as, the release of commensal derived MAMPs, pro-inflammatory cytokines, chemokines, Th17 and B cells cascades could lead to a loss in the intestinal barrier function. Therefore, these inflammatory responses may especially occur due to translocation of bacteria across the layer of the intestinal epithelium. To conclude, certain bacterial genres could especially exacerbate gut inflammation. such as, Prevotellaceae and Enterobacteriaceae. Also, the "bystander effect" may occur due to a decrease in the auto activation against self-antigens because of the loss of tolerance and lesser thresholds of the autoimmunity activation in the extra-intestinal tissue.

2.6 GUT MICROBIOTA METABOLIC PRODUCTS AND THEIR ROLE IN THE HUMAN HOST

The microbiota is responsible for fermentation and secretion of several metabolic products. Daily variations in food[146] contribute to the metabolite profile in plasma.[147] Consequently, a loss of certain microbial species can remove immune modulating metabolites that are necessary to maintain gut homeostasis. To name a few, some of the most relevant metabolites regulated and produced by the microbiota are short-chain fatty acids (SCFAs), bile acids (cholate, hyocholate and deoxycholate among others), choline metabolites (methylamine, dimethylamine), phenolic, benzoyl and phenyl derivatives (benzoic acid, hippuric acid), indole derivatives (N-acetyltryptophan, indolacetate), vitamins (vitamin K, vitamin B12, biotin), polyamines (putrescine, cadaverine), lipids (conjugated fatty acids, LPS, peptidoglycan) and others (D-lactate, formate, methanol).[143]

SCFAs regulate host immune responses. The most intensively investigated of all the metabolites secreted and fermented by the microbiota are short-chain fatty acids (SCFAs). SCFAs are chemical compounds made of carboxylic acid moiety and a small hydrocarbon chain. The most commonly studied SCFAs are acetic, propionic and butyric acids, which have two, three and four carbons in their chemical structure, respectively.[148] Members of the commensal microbiota,

particularly *Clostridioides* spp., produce SCFAs byproducts derived from the anaerobic fermentation of dietary fiber.[148] Importantly, SCFAs help to maintain Treg cell expansion, immunosuppressive function and overall intestinal homeostasis.[149] SCFAs have its most impact in the host immunity and metabolism.[149]

The gut microbiota-derived signal molecules are associated with multiple diseases and systemic disruption, suggesting that there is a cross-talk of the host-microbiota to extra intestinal organs.[149] For instance, acetate effects on the brain involve increasing satiety and neurogenesis so as decreasing blood brain barrier permeability.[149] That is, the effects on the brain have repercussion in the liver physiology increasing insulin sensitivity and the activated protein kinase (AMPK) activity and decreasing lipid storage and gluconeogenesis, hence, through receptors binding, acetate also reduce lipolysis and insulin-mediated fat accumulation.[149] Because of the translocation of bacterial products to distant organs, having a diverse microbiome is essential to maintain the vital functions of a healthy host since its contribution to the host's physiology is highly significant.[149]

Gut microbiota SCFAs production has a profound impact on host systemic immunity. SCFAs bind to G protein-coupled receptors (GPR41, GPR43, GPR109A). The binding of SCFAs to the GPCR produce effects such as increase in mucus, Tregs and sIgA production, enhancement of barrier integrity, inhibition of NF-kB and reduce T cell expression.[141]In particular, butyrate is the most studied of all the SCFAs. Colonocytes butyrate consumptions decrease inflammation through secondary increase of Treg cells.[150]Also, butyrate impacts the generation of dendritic cells and HDAC-related inflammation through binding to GPR109A.[150] Transportation of butyrate into the intestinal epithelial cells affect the metabolism and inhibition of the histone deacetylase activity (HDAC).[150] Acetate and propionate affect neutrophils through the receptor GPR43 so as the expansion of Treg cells.[148]Acetate coupling with GPR41 have an impact in the hematopoiesis of dendritic cells so as in decreasing the incidence of asthma. To add, the influence of SCFAs in the development and severity of infections has also been investigated in the clinical setting. For example, higher GPR43 RNA expression is associated with improved survival of patients with sepsis.[151] Figure 4. Illustrates how SCFAs contribute to the induction of pTreg cells. SCFAs entering dendritic cells act as inhibitors of histone deacetylase (HDACi) to suppress the expression of pro-inflammatory cytokines. They also directly act on naive T cells through GPR43 or the

upregulation of Foxp3 expression through HDAC inhibition.[38] *Figure 4 adapted from Honda K and Littman DR.*

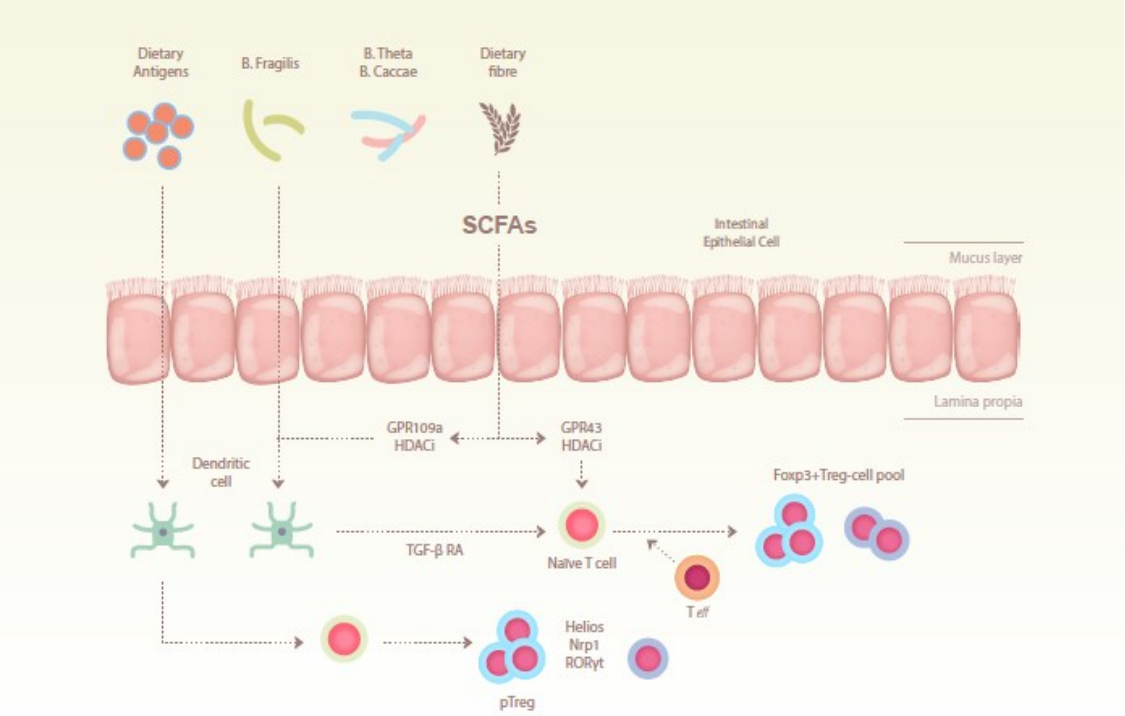

***Figure 4**. Adapted from Honda K and Littman DR.* Short-Chain Fatty Acids, microbiota and immunological mechanisms.

2.7 DISRUPTION OF GUT MICROBIOTA ALTERS HOST HOMEOSTASIS

The host maintains a homeostatic relationship with the gut microbiome. As previously stated, extrinsic factors such as, diet and antibiotics exposure could significantly alter the microbiota. Presently, studies are assessing the long-term consequences and impact of the microbiota dysbiosis in both molecular- and cultivation-based approaches. In addition, there is overwhelming evidence suggesting that the microbiota suffers ecological disturbances after antibiotic administration.[152,153] Therefore, diseases and pathophysiological alterations such as, inflammation, bacterial colonization, proliferation of pathogenic bacteria, abnormalities in metabolic processes and damage of the gut barrier lead to dysbiosis.

The microbiota is responsible for nutrient processing availability and has a profound impact in the human immune system. In case of gut dysbiosis, segmented filamentous bacteria like *Acinetobacter* spp., *Bacteroides fragilis* and Proteobacteria, increase their ability to permeate

and interact with the intestinal epithelium.[140] A direct bacteria-epithelium interaction has a profound impact on the immune system as bacteria could activate specific immunological pathways that promote inflammation cascades. For this reason, products of inflammation feed the expansion of colitogenic pathobionts implying that the "dysbiosis" in many diseases occurs as a consequence of inflammation.[154] Inflammation in the gut can directly alter microbial composition and function. Intestinal inflammation promotes the growth of certain facultative anaerobic bacteria while decreasing the growth of obligate anaerobes.[154,155] In a study, mice host with genetic predisposition develop inflammation after receiving a disrupted microbiota transplant from TNF^{delta} are mice with ileitis.[156] A loss in certain microbial species can remove immune modulating metabolites that are necessary to maintain gut homeostasis. For example, loss of the metabolite butyrate increases oxygenation in the lumen leading to aerobic luminal expansion of aerotolerant bacteria like *E. coli.*[157]

Bacterial microbiota members and antibiotic susceptibility. The use of antibiotics in excess might be contributing to the increase in diseases like obesity, irritable bowel disease, diabetes type 1, allergies, and asthma.[158] Consequently, because of the intensive use of antibiotics there has been a long-term persistence of antibiotic resistance genes in the gut microbiome. There are members of the microbiota bacterial community that present special susceptibility or resistance to antibiotics, thus, contributing to alterations in the microbiome associated with antibiotic use. Also, in the human host environment, antibiotic-resistant bacteria strains are able to survive even in selective pressure conditions.[159,160] In spite of the antibiotics impacting the human microbiota typically short-term, the impact period in the lack of diversity might extend long-term being even of major significance to the host's health.[159]

Lack of microbiota diversity and disturbances in the microbiome intestinal ecosystem are correlated with diseases and diseases susceptibility to infections. For instance, the frequent use of antibiotics may lead to diarrhea, alterations in carbohydrate metabolism and change in gastrointestinal physiology. An specific example is *Clostridium difficile,* this specie is an opportunistic pathogen that could proliferate as a result of alterations caused by antibiotic use that may lead to toxicity, henceforth, inducing colitis in the host.[161]Likewise, Crohn's disease has also been associated with the continuous use of antibiotics in early childhood.[162] In spite of the existing tools used to measure alterations in the microbiome composition, in order to understand how the microbial community and ecosystem is sustained and remains healthy we need further studies that

combine the understanding of the microbiome profile with physiological and clinical measures. All in all, the administration of antibiotics could alter the structure and function of healthy gut microbiota.

2.8 GUT MICROBIOTA IN CHRONIC DISEASES

Current literature says that there is a bidirectional relationship between disturbances in the gut microbiota and chronic diseases. For example, diseases such as liver cirrhosis, non-alcoholic steatohepatitis, coronary artery disease, cardiomyopathies and chronic kidney disease may be significantly impacted because of the disruption in the microbiome or vice-verse, that is, presence of the disease would cause the microbiome disruption.

Heart disease patients with atherosclerotic cardiovascular disease (ACDV) present similar buccal and gut bacteria, thus suggesting a migration bacterial hypothesis between the gut and the oral cavity.[163] For example, species like *Lactobacillus salivarius, Solobacterium moorei and Atopobium parvulum* were higher in patients with ACVD compared to healthy controls.[163] The microbiota in patients with heart disease secretes a compound known as trimethylamine (TMA). After trespassing liver metabolism, TMA becomes its active form as compound trimethylamine oxidase (TMAO), playing a role in the formation of the atherosclerotic plaque.[105,163] TMAO is cleared through glomerular filtration, which means that renal function plays a significant role in maintaining the levels of circulating TMAO, thus, affecting the vascular endothelium.[105,163] Moreover, the presence of *Eggerthella lenta* in heart diseases patients may have an enzymatic deactivating effect towards the cardiac drug digoxin[164,165] which may have an impact in the treatment of several heart conditions such as heart failure. What is more, *Lactobacillus salivarius, Solobacterium moorei*, *Atopobium parvulum and Eggerthella lenta* have been reported to be high in patients with ACVD.[164,165]

Dysbiosis in pre-transplant renal patients may be associated with systemic inflammation and the immunodeficiency of the host. Metabolic alterations in renal patients increase uremia levels favoring pathogenic overgrowth and dysbiosis.[166] That is, increased levels of Firmicutes, Proteobacteria and Actinobacteria are seen in chronic renal disease (CRD).[167]Likewise, levels of bacteria metabolite production of indoxyl sulfate, p-cresol, ammonia, urea and TMAO are increase in CRD,[167] therefore, impacting the human host renal function and the cardiovascular system. Some changes during CRD that may contribute to the bidirectional immunological relationship

between the host and the microbiota dysbiosis are increased production of proinflammatory cytokines, complement activation, impaired antigen presentation, B cells response and CD4+ / CD8 T cells ratio.[167]Additionally, there is also an increase in the translocation of microbiota metabolites during renal failure.

Patients with chronic liver disease present immune disturbances due to alterations in SCFAs metabolism. To begin, the gut microbiota is an important source of SCFAs for the host. For example, reduction of cancer cell proliferation is mediated through propionate in liver disease.[168] The effect of propionate in healthy livers, however, has yet to be studied. Another SCFAs playing a role in liver disease is butyrate. For instance, butyrate acts protecting the liver from progressing from early stages to non-alcoholic fatty liver disease[169–171]so as, ischemia/reperfusion syndrome.[169,172] Another study found that administration of butyrate through intravenous injection protects mice from ischemia/reperfusion injury.[169] Gut inflammation in liver diseases may occur due to microbiota products translocation. Diseases such as, alcoholic hepatic steatosis may progress rapidly to non-alcoholic steatohepatitis (NASH) because of the binding of the translocating bacterial metabolic products with toll-like receptors.[173] In addition, intestinal Reg3b and defensins have been found to be lowered in cirrhotic rats with ascites.[174] That is, intestinal Reg3b acts controlling for bacterial overgrowth in cirrhosis.[174] The low levels of defensins and Reg3b predispose the host to less protection against Enterobacteriaceae. Furthermore, cirrhosis also presents increase translocation of Gram-negative bacteria to the gut accelerating liver fibrosis.[173]

2.9 GUT MICROBIOTA IN TRANSPLANT PATIENTS

Solid organ transplantation (SOT) is a surgical procedure performed as curative treatment for most chronic and end-stages diseases affecting organs such as the lungs, heart, kidney, liver and pancreas, among others. First of all, transplantation restores the microbiota diversity increasing Proteobacteria and decreasing Firmicutes species relative abundance. Moreover, the defined host-microbiome metabolic axis represents a multidirectional chemical communication among specific cellular pathways, sub-ecologies and activities in the host.[163] The bidirectional interaction of the human microbiota with the host and its immune system has sparked significant interest given its potential impact in post-transplantation outcomes, including the prospect for generating novel preventative and therapeutic strategies to promote immune-tolerance and allograft rejection.

In kidney transplantation a study analyzing the microbiota in oral, urine, and stool samples was done before transplantation, at 1, and 6-months post-transplant. These changes in the kidney transplant recipients persisted over time after transplantation[27], and were associated with acute rejection, diarrhea, and urinary tract infection rates post-transplantation. Interestingly, there was a specific microbial Shannon diversity index correlation for each complication.[175] In mice models, anti-inflammatory effect of SCFAs has also been correlated with protection of induced ischemia-reperfusion in acute kidney injury.[176]

In liver transplantation, researchers from China collected fecal and blood samples from 111 liver transplant recipients and found significant disturbances in the gut microbiota. That is, altered levels of bacteria species such as *Eubacteria, Bifidobacterium, Faecalibacterium* and *Lactobacillus*, so as higher levels of *Enterococcus* and *Enterobacteriaceae* with the exception for *Enterococcus.*[91] In spite of the alterations found in the liver transplant, the levels of bacteria returned to normal after 6-months post-transplantation[94]. Another study found that there is an association with increased postoperative infections and a lack in microbiota diversity post-transplantation in liver.[44]

As previously mentioned, liver chronic disease is associated with microbiota dybiosis. For example, a recent report on recurrent primary sclerosing cholangitis was found to be associated with dysbiosis, however, recovery of the microbiota diversity was resolved after transplantation.[177,178] Variation of gut microbiota was found to predict early acute cellular rejection after liver transplantation in a rat model,[179]suggesting that a microbiota profile or alterations therein might hold promise as a predictor of acute rejection post-transplantation. The gut microbiota has been linked to the development of new-onset diabetes mellitus post-liver transplantation through alterations of host metabolic homeostasis by as yet unclear gut-liver axis mechanisms.[180]

In 21 lung transplants recipient, the microbiota community was found to be altered in the analyses of bronchoalveolar lavage (BAL). Bacterial organisms present in BAL, appeared to be a heavy load and decreased in microbial diversity.[181]In another study BAL specimens from 57 lung transplant patients presented higher rates of bronchiolitis obliterans and major complications, such as allograft rejection.[182] In such cases, restoration of the lung microbiota pre-transplant seemed to protect against bronchiolitis obliterans.[182] In small intestinal transplantation, microbiota dysbiosis suggested risk for allograft rejection. For instance, in small intestinal transplant recipients

Firmicutes and *Lactobacillales* species were significantly reduced and Proteobacteria was increased in ileal effluents.[183] Therefore, the overall findings of this study indicate that the microbiota profile may serve as a diagnostic biomarker for risk of allograft rejection in small intestine transplantation.[183]

These findings suggest that monitoring the gut microbiome peri-transplantation can provide a potentially useful surrogate marker for outcomes post-transplantation, and manipulation of the microbiome may help realize improved outcomes.

2.10 GUT MICROBIOTA PHENOTYPE IN TRANSPLANT PATIENTS AND FUNCTIONAL IMPLICATIONS IN TRANSPLANT PATIENT-OUTCOME

Most of the research and evidence related to the gut microbiota and transplant patients focuses in the understanding of microbiota phenotype composition and alterations within the gut bacterial ecosystem and community. As a result, the current understanding of the implications and association between transplant outcome and the microbiota mostly relies on information about the microbiome phenotype profile of transplant patient. Post-transplantation success is reliant on many factors previously mentioned such as, interaction between the use of antibiotics, immunosuppression, chronic diseases, transplant complications and rejections are combined factors that have an impact in the transplant patient outcome. Altogether, these factors may disrupt the microbiome composition in the transplant patient population. Although evidence suggests that dysbiosis in liver, heart, kidney and lung patients is resolved in a large percentage of transplanted patients post-transplantation,[184] a percentage of patients undergoing transplantation does not recover their heathy microbiota post-transplant. However, the exact mechanisms through which recovery of the healthy microbiota in transplant patients is achieve are unknown.

Transplant patients are a vulnerable group receiving multiple types of treatments after transplant. The use of prophylactic antibiotic and immunosuppressant therapy may have an impact in the gut microbiota composition. For example, in a pre-clinical mice models done by Rey et al.,[185] the effects in the use of post-transplant antibiotics and microbiota alterations were tested to see if there was an association with acute rejection post-aortic interposition grafting. In this study, researchers found that disruptions of the microbiota due to antibiotic use might exacerbate the immune response causing acute vascular rejection.[185] Evidence from studies in liver transplant suggests that patients recover their microbiota diversity post-transplant.[184,185]

There seems to be no changes in the relative abundance of taxa pre- vs post-liver transplantation. However, a reduction in pathogenic genera such as *Enterobacteriaceae* and an increase in beneficial autochthonous taxa (*Ruminococcaceae and Lachnospiraceae*) was found post-liver transplant. In addition, this study mentioned an improvement in cognitive function post-liver transplant potentially associated with the improvement in the microbiota diversity due to the gut-liver-brain microbiota axis.[184]Currently, the evidence on the impact of the microbiota post-transplantation is scarce. All in all, we know that microbiota dysbiosis may affect functionality of the host, importantly enough pursue further research to identify the clinical implications of the microbiota in the transplant patient-outcome.

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CHAPTER 3

METHODS

3.1 PATIENTS AND METHODS

We performed a retrospective cohort analysis of all adult patients (≥18 years old) who underwent liver transplantation for chronic liver disease between 1st September 2014 and 31st December 2017 at the University of Alberta Hospital in Edmonton, Alberta, Canada. This study was conducted to evaluate the association of VRE colonization with liver transplant mortality at 2-years as our primary endpoint and VRE association with the secondary endpoints for the first 6-month post-transplantation. The liver transplant program at the University of Alberta Hospital started in 1989. The University of Alberta hospital maintains a computerized database using the Organ Transplant Tracking Record (OTTR, HKS Medical Information Systems, and Omaha, Nebraska, USA) dedicated to track and follow solid organ transplants since 1995 and used at the University of Alberta Hospital since 2012. This study was approved by the University of Alberta Health Research Ethics Board (HREB_Pro00082528)

Subject identification, interventions and operational definition. Liver transplant patients were initially identified using OTTR. Data gathered for this study included patients' age, gender, indications for OLT, serum parameters, severity of liver disease indexes (MELD), donor transplant, CMV donor-recipient status, infection complications, acute rejection, immunosuppression, follow-up, and survival status that were retrieved from OTTR. VRE status was retrieved from Alberta Health Services ProvLab database**.**

Rectal swabs for VRE colonization were performed in all liver transplant patients before undergoing liver transplantation. VRE colonization was defined as positive if present in culture-based screening after performing rectal swab. Surgical prophylaxis consisted of Imipenem or Meropenem plus linezolid for 24 hours post-surgery. The University of Alberta Hospital liver transplant program indications and contra-indications for orthotopic liver transplantation (OLT) and immunosuppression regimen was used to considered performing of liver transplant. The Model for End Stage Liver disease (MELD), a measure use to calculate severity of liver disease to triage patients undergoing liver transplant in the waiting list. MELD score was calculated by gathering bilirubin (mg/dl), INR and serum creatinine (mg/dl) values pre-transplantation. Acute kidney injury (AKI) was defined as an increase in basal creatinine x1.5 times during the first 30

days post-transplantation. Clinically significant infections were microbiologically confirmed infections requiring systemic antibiotic and hospital admission or appearing during admission in the first 6-months after transplantation. The reported infections included fungus, bacteria and/or virus.

Acute rejection was biopsy-proven rejection according to Banff criteria at 6-months after transplantation. Clinically, the classification of Ischemia reperfusion injury (IRI) in most clinical trials include 3 groups defined according to the maximum level of aspartate aminotransferase (AST) in the first 72 hours post-transplant: Group 1 < 600 IU/L; Group 2: 601-2,500 IU/L; Group: 3 2,501-5,000 IU/L.[1–3]

Health clinical outcome included patient and graft survival status, follow-up and causes of death. The primary cause of death was used to calculate Kaplan-Meier survival analysis. Patient survival and mortality were primary endpoints. Acute rejection, clinically significant infections, ischemia reperfusion injury and acute kidney injury were secondary endpoints.

3.2 STATISTICAL ANALYSIS

Categorical variables were presented as proportions and continuous variables as mean and standard deviation (SD) if normally distributed, or median and inter-quartile range (IQR), if non-normally distributed. Categorical variables were compared using Chi-square (or Fisher exact test if necessary). Continuous variables were compared by Student's T test or or by Mann -Whitney U test depending on normal distribution. For variables depending on time, Kaplan-Meier survival analysis was performed, and arms were compared by log-rank test. Multivariable analysis was performed by Cox-regression analysis, entering clinically relevant covariables and variables with a level of significance < 0.1 in the univariate analysis. For all performed tests, a p-value <0.05 in a two-sided test was considered statistically significant.

All statistics were calculated with IBM SPSS Statistics software (Chicago, IL) version (26). Categorical variables are summarized as percentages.

3.3 REFERENCES

1. Busuttil, R. W. *et al.* rPSGL-Ig for Improvement of Early Liver Allograft Function: A Double-Blind, Placebo-Controlled, Single-Center Phase II Study†. *Am. J. Transplant.* **11**, 786–797 (2011).
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3. Khan, A. W., Fuller, B. J., Shah, S. R., Davidson, B. R. & Rolles, K. A prospective randomized trial of N-acetyl cysteine administration during cold preservation of the donor liver for transplantation. *Ann. Hepatol.* **4**, 121–126 (2005).

CHAPTER 4

RESULTS

4.1 PRE-TRANSPLANT COHORT

Cohort pre-transplant characteristics. During the study period, we identified 351 adult liver transplants that were performed at the University of Alberta Hospital and retrospectively reviewed. Of these, 8 patients were missing information on pre-transplant VRE colonization and were, therefore, excluded from the study. The final cohort included 343 liver transplant recipients. Median age was 56.5 years (IQR 45.5-61.4) and 231 (67%) were male. Pre-transplant cohort according to age groups are shown in figure 5 below. Most common indication for liver transplantation included viral hepatitis (35%), alcohol (15%), and primary sclerosing cholangitis (13%) shown in figure 6.

Figure 5. Pre-transplant cohort according to age group

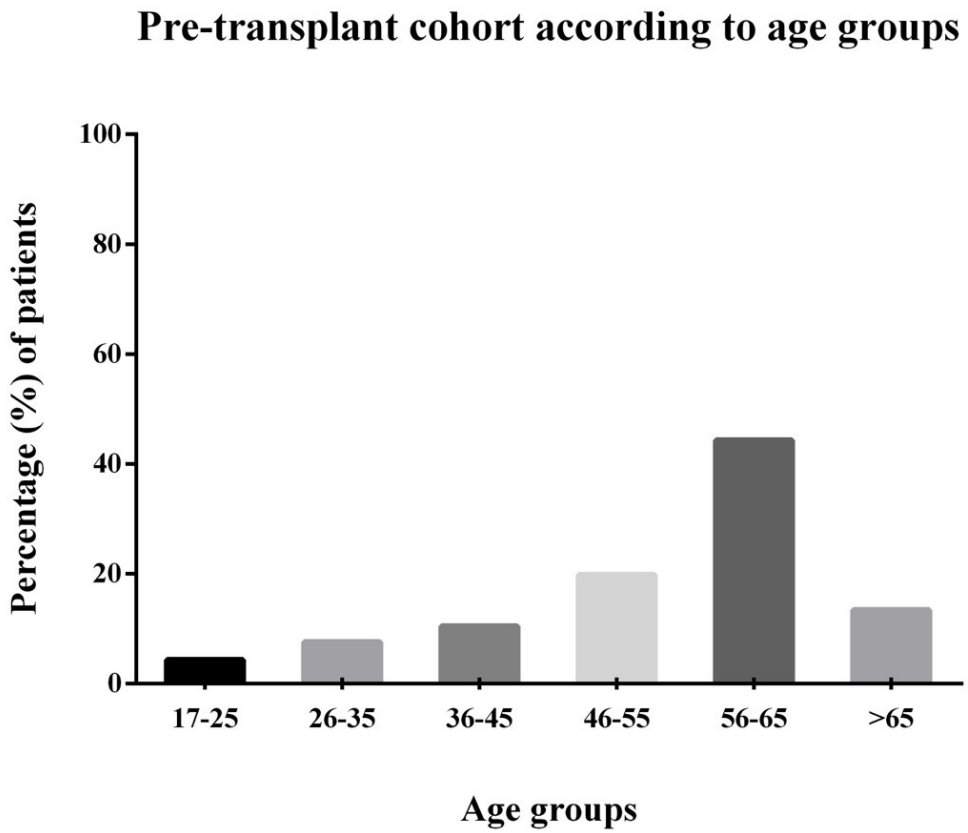
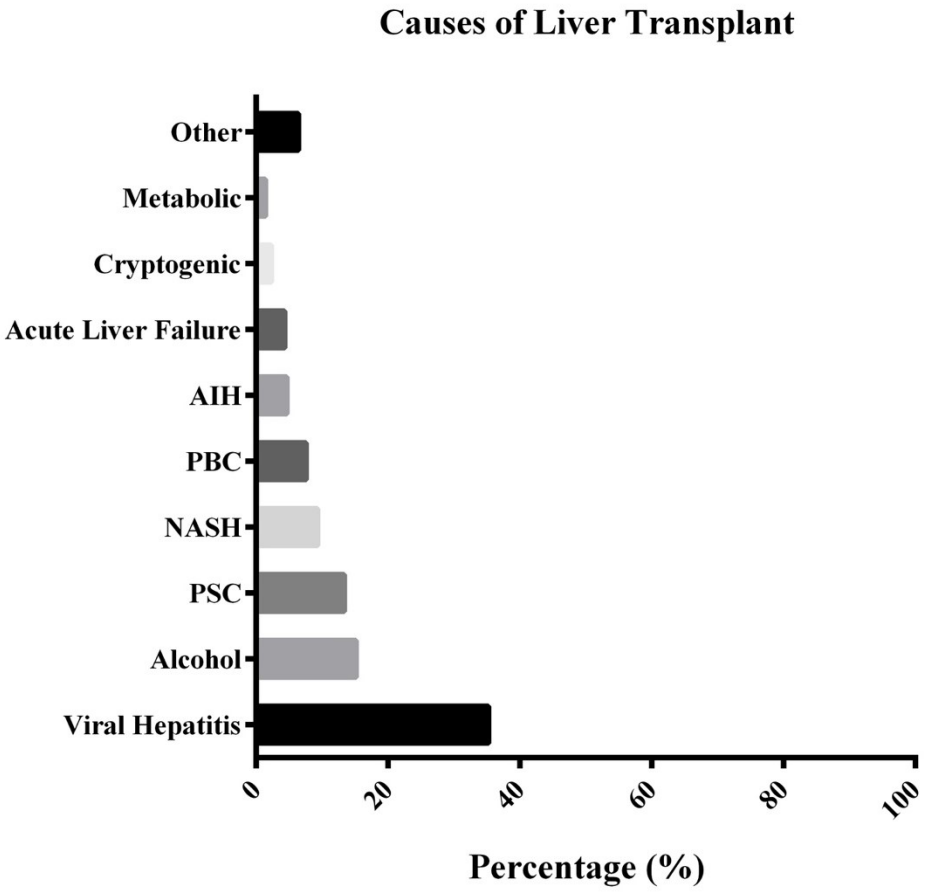


Figure 6. Causes of Liver Transplant



Model of End-Stage Liver Disease (MELD) median biochemical score was 18 (IQR 12-25). Median creatinine pre-transplantation was 75 (umol/l) (IQR, 61-98). At transplant, donor CMV serology: Positive 188/343 (55%). CMV Negative 155/343 (45%). Recipient CMV serology: Positive 201/343 (59%). CMV Negative 142 (41%). CMV donor-recipient pre-transplant prevalence was, D+/R- mismatch 81 (24%), D+/R+ 106 (31%), D-/R+ 94 (27%), D-/R- 61 (18%). Prevalence of VRE colonization pre-liver transplant was 19.8 % (68/343). Table 1. shows a summary of the pre-transplantation cohort characteristics.

Table 1. Pre-transplant cohort characteristics

	Whole cohort (n= 343)
Gender	
Male	231(67)
Female	112 (33)
Age	
Mean (SD)	52.4 (12.12)
Median (IQR)	56.5 (45.5-61.4)
MELD Score	
Mean (SD)	19.74 (9.642)
Median (IQR)	18.0 (12.0-25.0)
Donor CMV serology	
Positive	188 (55)
Negative	155 (45)
Recipient CMV serology	
Positive	201(59)
Negative	142(41)
CMV Donor/Recipient	
D+/R-	81 (24)
D+/R+	106 (31)
D-/R+	94 (27)
D-/R-	61 (18)
VRE colonization (Pre-Tx)	68 (19.8)

Table 1. Number between parentheses represent percentage (%) unless otherwise stated.

4.2 POST-TRANSPLANT COHORT CHARACTERISTICS

Ischemia-reperfusion injury grade III or IV was seen in 27 (8%) patients of the cohort. Median creatinine peak post-transplant was 128 (umol/l) (IQR, 81-184). The incidence of acute kidney injury within the first 30 days post-transplant was 189/343 (55%). Post-transplant incidence of VRE colonization during the first 90 days was of 24/275 (8.7%). Main induction treatment was basiliximab 322/343 (94%) and 3% received thymoglobulin. Acute rejection within 6-months post-transplantation was of 27 (8%). Increased immunosuppression was the preferred rejection therapy 22/37 (59%). Prednisone was administered in 16% of the acute rejection cases.

Infections post-transplant. Clinically significant infection incidence at 6-months (non CMV) was of 69 (20%). Incidence of bacteremia cases was of 14 (4%) and of VRE invasive infections was 7 (2%). Clinically significant CMV infection was of 39 (11%). Most common cause of bacterial infection was *Clostridium difficile* 15/77 (19.48%), of viral infections was CMV 35/53

(67.31%) and Candida was the most common fungal infection. Invasive VRE infection accounted for 8/77 (10%) of the bacterial infections post-transplant. Lastly, VRE colonization 90 days post-transplantation was identified in 24/275 (8.7%). Table 2 below summarizes the characteristics of infections post-transplant.

Table 2. Characteristics of infections post-transplant

Whole Cohort	n=343 (%)
Total Infections at 6 months	115 (34)
CMV disease	
Positive	41 (12)
Negative	301 (88)
CMV Infections	
Mean (SD)	144555.9661 (982752)
Clinically significant CMV Infections	
Mean (SD)	243637.7179 (1206067)
CMV tissue invasive disease	2/343 (0.6)
Number of CMV Colitis	1
Number of CMV Esophagitis	1
Bacterial Infections	
Clostridium difficile	15/77 (19.48)
Escherichia coli	8/77 (10.39)
Staphylococcus	6/77 (7.79)
Enterococcus Non-VRE	14/77 (18.18)
Enterococcus VRE	8/77 (10.39)
Enterobacter	3/77 (3.9)
Klebsiella pneumoniae	7/77 (9.09)
Mixed-Cultured	8/77 (10.39)
Other	8/77 (10.39)
Viral Infections	
CMV	35/53 (67.31)
HCV recurrence	12/52 (30.77)
Other	1/52 (1.92)
Fungal Infections	
Candida	4/4 (100)
VRE colonization (Post-Tx) - 90 days	24/275 (8.7)
VRE Invasive	7/343 (2.04)

Table 2. Number between parentheses represent percentage (%) unless otherwise stated.

Mortality. Major causes of mortality included infection (20%), chronic rejection (20%) and others (24%). Mortality during the first year occurred in 25 (7.3%) liver transplant recipients. Mortality until the latest day of follow-up was 41/343 (12%). Table 3 shows a summary of all mortality causes in the liver transplant recipients.

Table 3. Causes of mortality in the liver transplant recipients

Whole Cohort n=343	(n/total)	(%)
Mortality	41/343	12%
Causes of Mortality		
Cardiovascular	4/41	12%
Cardiac arrest	3/4	75%
Cardiogenic Shock	1/4	25%
Malignancy	7/41	17%
Abdominal	1/7	14%
Gastric Adenocarcinoma	1/7	14%
HCC	2/7	29%
Metastasis	3/7	43%
Infections	8/41	20%
Septicemia	6/8	75%
Bacterial	5/6	83%
Fungal	1/6	16%
Multiple Infections	1/8	12%
HCV recurrence	1/8	12%
Chronic rejection	8/41	20%
Graft failure	8/8	100%
Recurrent Primary disease	3/41	7%
Other	11/41	24%

4.3 VRE VS NON-VRE COLONIZED LIVER TRANSPLAN RECIPIENTS

The characteristics of the main variables between VRE colonize and non-colonized patients is summarized in Table 4. Our whole cohort included 343 liver transplant patients, for which 68 were colonized with VRE vs 275 non-VRE colonized. Immunosuppression therapy was Tacrolimus and Mycophenolate mofetil (MMF) accounting for treatment in 91% of the whole cohort. Importantly, the Model of End-Stage Liver Disease (MELD) was found to be

significantly different between the two groups as described as the Median MELD (IQR), which was higher for the VRE colonized 24 (18-29) in comparison to the non-VRE 17 (12-24); P<.001.

Other variables included in the study presenting no statistical significance were: Mean age of VRE and non-VRE colonization was 50.2 (13.5%) vs 52.9 (11.7%); p=0.092. Male gender for VRE colonized was 40 (59%) vs non-VRE colonized 191 (70%); p=0.094. Most common reason for transplant in both VRE and non-VRE colonized was 35% and 21 % vs 23% and 39%, for immune and viral cause, respectively. Median days of admission 21(16-49.5) vs 19 (12-32); p=0.024 for VRE vs non-VRE colonized.

Infections VRE vs non-VRE colonized described as CMV serostatus for VRE colonized was, CMV D-/R- 10 (15%), D-/R+ 24 (35%), D+/R+ 16 (23%), CMV D+/R- mismatch 18 (27%) and for non-VRE colonized was, CMV D-/R- 51 (19%), D-/R+ 70 (25%), D+/R+ 91 (33%), CMV D+/R- mismatch 63 (23%); p=0.235. CMV infection was 12 (18%) vs 47 (17%); p=0.913 for VRE vs non-VRE colonized. Median peak of CMV viral load for VRE colonized was 3222 (1192-6665) and for non-VRE colonized was 2048 (1092-5050); p=0.522. VRE vs non-VRE colonized for clinically significant bacterial/fungal infection was of 21 (31) vs 57(21); p=0074, for bacteremia/fungemia cases was 4 (6%) vs 10 (4%); p=0.49 and for invasive VRE infection was of 2 (3%) vs 5 (2%); p=0.629, respectively.

4.4 CLINICAL CHARACTERISTICS, PRIMARY AND SECONDARY ENDPOINTS OF VRE VS NON-VRE COLONIZED

To assess kidney function, we used values of median creatinine pre-transplant and median peak of creatinine post-transplantation. Median creatinine pre-transplant was 85 (65-108.5) vs 74 (60-96.5); p=0.069 for VRE colonized vs non-VRE colonized, respectively. Median peak of creatinine post-transplant 158 (113-192.5) vs 123 (78.5-175.5) for VRE vs non-VRE colonized showed statistical significance (p=0.004) with a higher median peak of creatinine post-transplant for the VRE colonized. Acute kidney injury was also seen in 66% VRE colonized patients' vs 54% non-VRE colonized. Lastly, acute rejection was seen in 8(12) vs 29(11); p=0.779 of VRE vs non-VRE colonized. Mortality in VRE colonized was of 10(15) vs 31(11) for non-VRE colonized; p=0.435. Table 4. next page shows all patient's demographics and clinical characteristics between the VRE colonized and non-VRE colonized groups.

Table 4. Patient's demographics and clinical characteristics

Variables	VRE colonized N=68	Non VRE colonized N=275	p Value*
Mean age (SD)	50.2 (13.5)	52.9 (11.7)	0.092
Male sex	40 (59)	191 (70)	0.094
Median MELD (IQR)	24 (18-29)	17 (12-24)	<0.001
Reason for transplant:			
● Viral	14 (21)	107 (39)	0.054
● NASH	8 (12)	24 (9)	
● Alcohol	12 (17)	41 (15)	
● Immune	24 (35)	63 (23)	
● Other	10 (15)	40 (14)	
Fulminant liver failure	2 (3)	13 (5)	0.744
CMV serostatus			
● CMV D-/R-	10 (15)	51 (19)	0.235
● CMV D-/R+	24 (35)	70 (25)	
● CMV D+/R+	16 (23)	91 (33)	
● CMV D+/R-	18 (27)	63 (23)	
Immunosuppression			
● Tacrolimus	62 (91)	250 (91)	0.945
● Cyclosporine	1 (2)	6 (2)	>0.999
● Sirolimus	16 (24)	73 (27)	0.611
● MMF	62 (91)	250 (91)	>0.999
Ischemia-reperfusion			
● Median AST (72h)	544 (296-1,041)	538 (282-1,020)	0.874
● Ischemia-reperfusion ≥ 3	6 (9)	21 (8)	0.801
Kidney function:			
● Median creatinine pre-transplant	85 (65-108.5)	74 (60-96.5)	0.069
● Acute kidney injury (30 days)	45 (66)	148 (54)	0.066
● Median peak creatinine post-transplant	158 (113-192.5)	123 (78.5-175.5)	0.004
CMV infection:			
● CMV infection	12 (18)	47 (17)	0.913
● Median peak CMV viral load	3222 (1192-6665)	2048 (1092-5050)	0.522
HCV recurrence	2 (3)	13 (5)	0.744
Bacterial/fungal infections			
● Clinically-significant infection	21 (31)	57 (21)	0.074
● Bacteremia/fungemia	4 (6)	10 (4)	0.49
● Invasive VRE infection	2 (3)	5 (2)	0.629
Median days of admission	21 (16-49.5)	19 (12-32)	0.024
Acute rejection	8 (12)	29 (11)	0.779
Mortality	10 (15)	31 (11)	0.435

4.5 KAPLAN-MEIER ANALYSIS

27 patients without VRE colonization at baseline acquired VRE post-transplant (27/275, 9.8%). VRE post-transplant in non-colonized patients at baseline occurred at a median of 8 days (IQR 4-44.5 days).

Primary endpoint. Probability of survival at 2-years was not significantly different between VRE-colonization at baseline and non-colonized patients; p=0.215 (Figure 7).

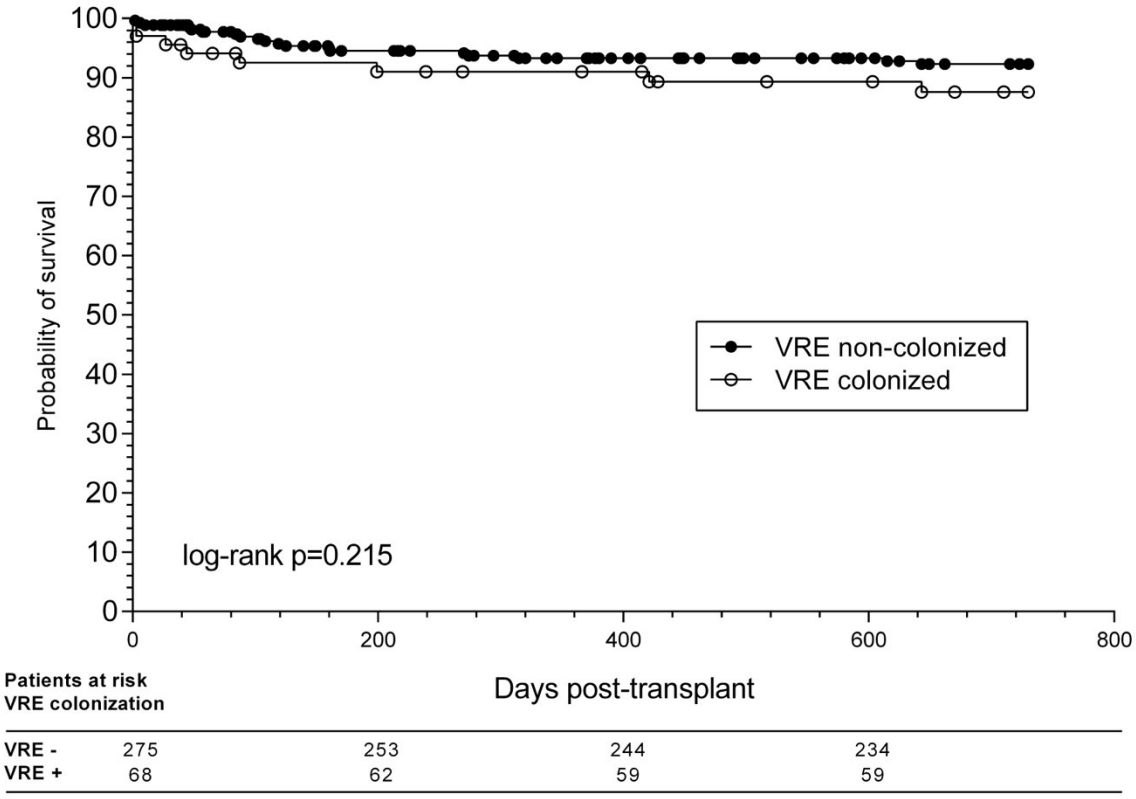


Figure 7. *Kaplan-Meier survival analysis at baseline VRE colonization (Log Rank test P = 0.215).*

Secondary endpoints. Percentage-free of clinically significant infections including bacteria, fungal and viral showed non-significant difference at baseline between the VRE colonized and non-VRE colonized; p=0.090 at 6-months post-transplant (Figure 8). Percentage-free of acute rejection was non-significant at baseline between the VRE colonized and non-VRE colonized; p=0.605 at 6-months after transplant (Figure 9). Percentage-free of acute kidney injury for VRE baseline colonization log rank curve was statistically significant between the VRE colonized and non-VRE colonized groups; p=0.009 at 30 days post liver transplantation (Figure 10).

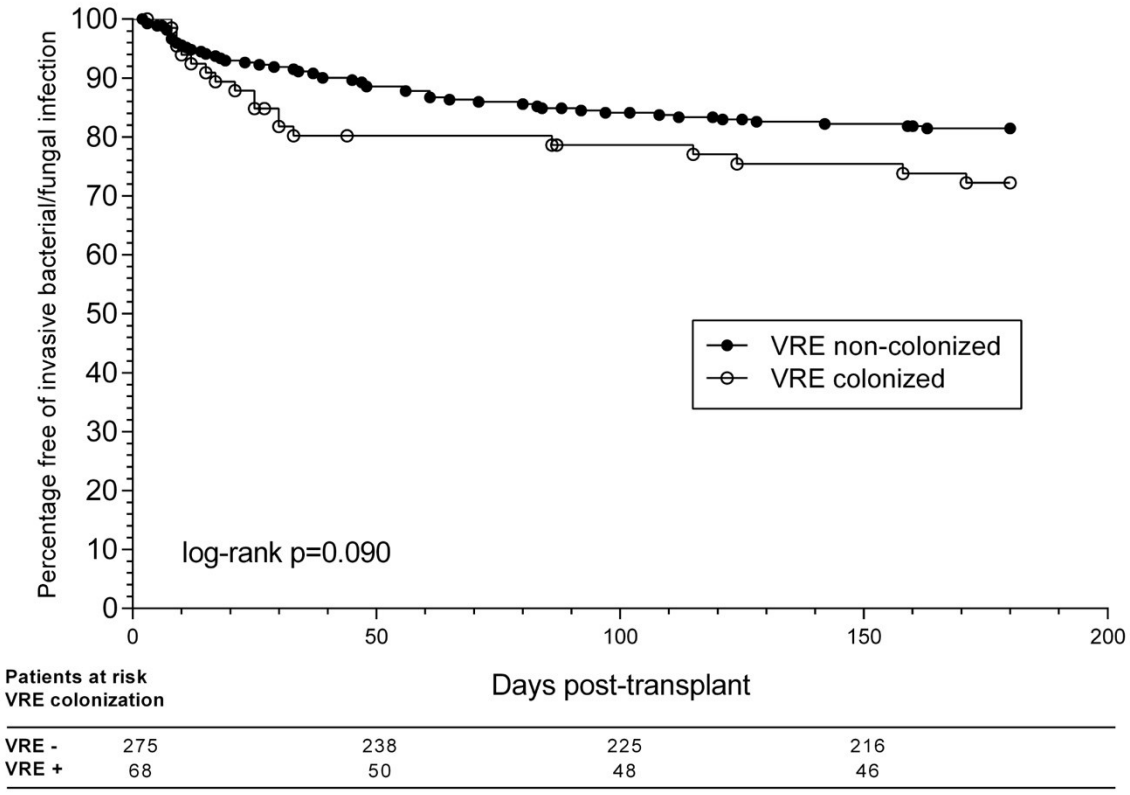


Figure 8. *Kaplan-Meier analysis of invasive bacterial/fungal infection at baseline VRE colonization (Log Rank test P = 0.090).*

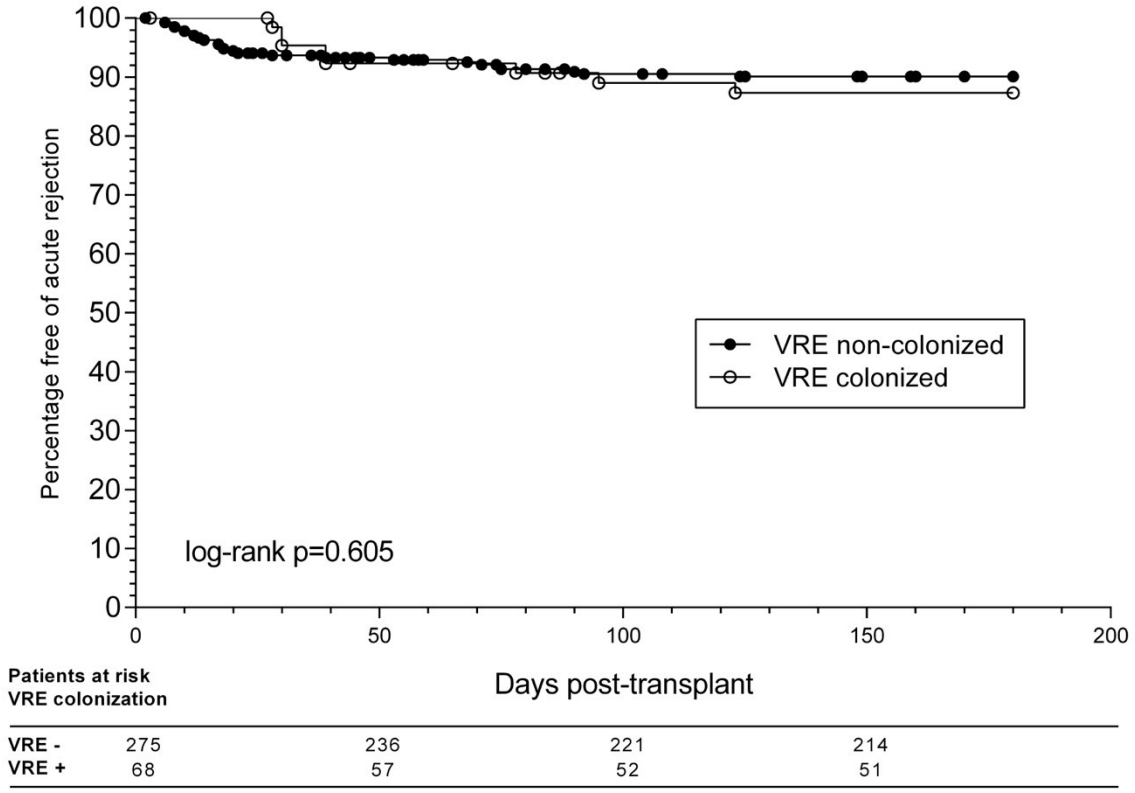


Figure 9. *Kaplan-Meier analysis of rejection at baseline VRE colonization (Log Rank test P = 0.605).*

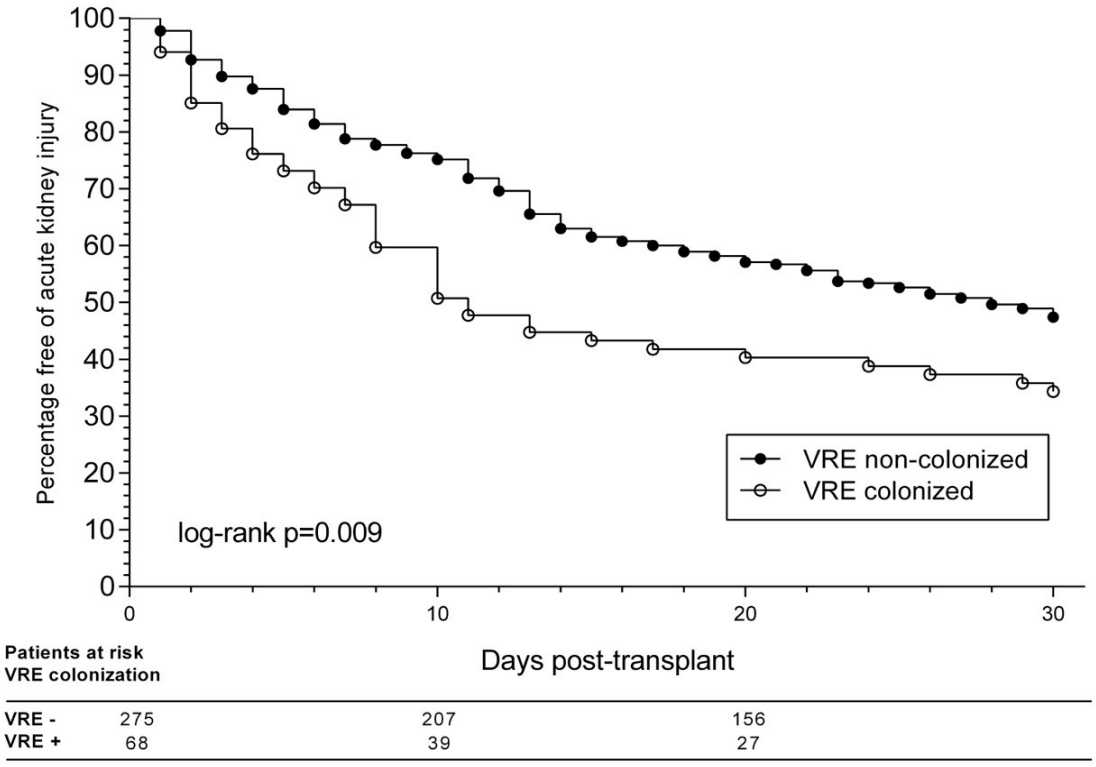


Figure 10. *Kaplan-Meier analysis of acute rejection at baseline VRE colonization (Log Rank test P = 0.009).*

4.6 MULTIVARIATE ANALYSIS

We performed a multivariate analysis to identify the independent variables associated with the development of acute kidney injury at 30 days post-transplant and reported the hazard ratio of adjusted by age, gender, MELD score, reason for transplant and VRE colonization at baseline. The risk of acute kidney injury at 30 days was not associated with age 52.8 versus 51.9, HR (1.005, 95% CI: 0.991-1.020; p=0.448), gender 131 (56%) versus 58 (51.8%), HR (0.774, 95% CI: 0.553-1.083; p=0.135). Mean MELD at transplant for patients with AKI (20.18) versus non-AKI (19.2), HR (1.016, 95% CI: 0.999-1.033; p=0.064) and reason for transplant (see details in table 5) were not significantly higher for acute kidney injury at 30 days. Of the 68 VRE colonized patients at baseline AKI 45 (66.2%) versus 144 with no AKI 144 (52.4%), the VRE colonized were at higher risk (1.610, 95% CI: 1.127-2.299; p=0.009) for acute kidney injury at 30 days post-transplantation.

Table 5. Multivariate analysis at the risk factors for acute kidney injury at 30 days

Variable	Category	n	Acute kidney injury (30 d)	aHR (95% CI)	p
Mean age at transplant	AKI	189	52.8	1.005 (0.991-1.020)	0.448
	No AKI	154	51.9		
Male sex	Yes	231	131 (56.7%)	0.774 (0.553-1.083)	0.135
	No	112	58 (51.8%)		
Mean MELD at transplant	AKI	189	20.18	1.016 (0.999-1.033)	0.064
	No AKI	154	19.2		
Reason for transplant:					
· **Viral**	Number	121	73 (60.3%)	1	-
· **NASH**		32	12 (37.5%)	0.451 (0.242-0.840)	0.012
· **Alcohol**		53	31 (58.5%)	0.822 (0.533-1.268)	0.375
· **Immune**		87	50 (57.5%)	0.913 (0.599-1.391)	0.671
· **Other**		50	23 (46.0%)	0.732 (0.424-1.263)	0.262
VRE colonization at baseline	Yes	68	45 (66.2%)	1.610 (1.127-2.299)	0.009
	No	275	144 (52.4%)		

We performed a Cox-regression multivariate analysis to identify the independent variables associated with mortality at 2-years and reported the hazard ratio of adjusted by age, gender, MELD score, reason for transplant and VRE colonization at baseline. The risk of mortality at 2-years was not associated with age 52.8 versus 52.43, HR (1.012, 95% CI: 0.979-1.047; p=0.47), gender 18 (7.8%) versus 11 (9.8%), HR (0.946, 95% CI: 0.462-2.110; p=0.894). Mean MELD at transplant for patient death (20.9) versus alive (19.63), HR (0.997, 95% CI: 0.417-2.143; p=0.888) and reason for transplant (see details in table 6) were not significantly at a higher risk for mortality. Of the 95 VRE colonized patients at baseline death 12 (12.6%) versus 248 alive 17 (6.9%), the VRE colonized showed a tendency for a higher risk at 2-years after transplantation (1.974, 95% CI: 0.890-4.378; p=0.094).

Table 6. Multivariate analysis at the risk factors at 2-years mortality

Variable	Category	n	Death	aHR (95% CI)	p
Mean age at transplant	Death	29	52.08	1.012 (0.979-1.047)	0.47
	Alive	314	52.43		
Male sex	Yes	231	18 (7.8%)	0.946 (0.462-2.110)	0.894
	No	112	11 (9.8%)		
Mean MELD at transplant	Death	29	20.9	0.997 (0.417-2.143)	0.888
	Alive	314	19.63		
Reason for transplant:	Number	121	9 (7.4%)	1	-
· **Viral**		32	3 (9.4%)	1.162 (0.307-4.402)	0.825
· **NASH**		53	3 (5.7%)	0.726 (0.193-2.729)	0.636
· **Alcohol**		87	4 (4.6%)	0.580 (0.161-2.089)	0.507
· **Immune**		50	10 (20%)	3.216 (1.090-9.488)	0.034
· **Other**					
VRE colonization*	Yes	95	12 (12.6%)	1.974 (0.890-4.378)	0.094
	No	248	17 (6.9%)		

CHAPTER 5

DISCUSSION AND CONCLUSION

Vancomycin-resistant Enterococci (VRE) is a prevalent antibiotic resistance bacterium known for its ability to colonize the gut potentially acting as a surrogate marker of microbiota dysbiosis. That is, VRE gut colonization may suggest the presence of microbiota dysbiosis. For this reason, VRE colonization might be responsible for disturbing the microbiota metabolism, metabolite and by-products production involved in inflammation, energy homeostasis, immunity, cell proliferation and apoptosis. VRE colonization is oftentimes seen in patients with chronic liver diseases and, therefore, in the liver transplant. Liver transplant patients are at a major risk of VRE colonization due to their exposure to antibiotics[1–4] and prolonged hospital admissions. VRE colonization rates in liver pre-transplant are 11.9%, and 16% in post-transplant patients.[5] In addition, there are a number of studies suggesting that mortality rate is higher in the liver transplant due to VRE colonization and infection.[6–8] Consequently, VRE colonization itself might be a risk factor associated with liver transplant complications and for worse health outcomes because of the implications in the microbiota gut dysbiosis.

5.1 SUMMARY OF RESULTS

We found that the prevalence of VRE colonization in patients undergoing liver transplantation is 68 (19.8%). In addition, 27 (9.8%) of patients at risk acquired VRE colonization after transplantation. We found that VRE colonized liver transplant patients had a higher Model of End Stage Liver Disease (MELD) score and had higher risk of developing acute kidney injury post-transplant at 30 days follow-up. First, we found that MELD score in VRE colonized liver transplant patients was higher (worse) compared to non-VRE colonized. Second, as we evaluated kidney function, we found that there was an increase in the median peak of creatinine post-transplantation in the VRE colonized compared to the non-VRE colonized. Our Kaplan-Meier analysis also showed that VRE colonized patients were less likely to be percentage-free of acute kidney injury at 30 days post-transplant compared to the non-VRE colonized. Furthermore, results from our multivariate analysis showed that patients colonized with VRE at baseline had a higher risk of presenting acute kidney injury at 30 days. Finally, even though we found no significant difference in survival between the VRE colonized compared to

the non-VRE colonized at 2-years after transplantation, our multivariate analysis adjusted by aged showed that patients pre-liver transplantation VRE colonized at baseline showed a tendency for higher risk of mortality than the non-VRE colonized.

5.2 DISCUSSION

VRE colonization could be consider a surrogate marker of microbiota dysbiosis predisposing liver transplant VRE colonized patients to develop more complications and worse clinical outcomes post-transplant. In our study, patients with VRE colonization had worse MELD scores and, therefore, were more likely to present complications such as acute kidney injury and a tendency for a higher mortality risk post-transplantation.

Effect of VRE in Liver transplant. Vancomycin-resistant Enterococci has been associated with higher mortality rate and complications risks in the liver transplant.[8–10] VRE is consider a nosocomial colonizing bacterium because of its ability to colonized during extensive hospitalization stays and ICU admissions. The risk for VRE colonization in the liver transplant patient is associated to the use of antibiotics. For example, in a study done by Ubeda et al. the presence of a dominant VRE population in the gut microbiome was increased in mice treated with antibiotics.[11] This study also showed that patients undergoing allogenic hematopoietic stem cell transplantation proceeded to develop bloodstream infection, if the gut was dominated by VRE.[11] When VRE colonizes and predominates the gut microbiota ecosystem, VRE colonization may lead to gut dysbiosis. Consequently, negative effects in the host's immunity and homeostasis may compromise patients' health outcomes.

The microbiota is responsible for secreting metabolites that interact with the host immune system and homeostasis. VRE colonization may disrupt the microbiota as it produces dysbiosis in the gut, therefore, affecting bacteria metabolite production. Since metabolic products of the microbiota affect kidney function, acute kidney injury (AKI) complication seen in the liver transplant may occur as a consequence of the microbiota dysregulation.[12]

As revealed by researchers, there are two mechanisms that explain the reason microbiota dysregulation may lead to AKI. Ateration of the production in short chain fatty acids (SCFAs) and the Trimethylamine-N-Oxide (TMAO) by-product metabolism.[12] For example, SCFAs play a role modulating G-coupled protein receptors signaling, hence, their implication in the inhabiting of cell proliferation, T-cell differentiation, homeostasis and immunity of the blood

vessels and kidneys.[13–15] Likewise, the Olfr78 receptor of SCFAs seems to be involved in modulating the role and function of the blood pressure.[16] As SCFAs play many roles in energy homeostasis, immunity and inflammation regulation, its role involving signalling of GPCR might be the most specific rationale for its pathophysiological implications in acute kidney injury.

Another important metabolic explanation correlating AKI and microbiome dysbiosis is the TMAO by-product through the TGFB/Smad3 signaling pathway[17]. TMAO is an amine-oxide gut microbiota specifically synthetized by-product derived from trimethylamine (TMA) from the dietary nutrition of choline and carnitine.[18] Current literature suggests that high levels TMAO are associated with cardiovascular and renal complications such as, chronic kidney disease.[19–21] The mechanism by which accumulated and high levels of TMAO microbiota by-product may be implicated in acute kidney injury is through promotion of renal interstitial fibrosis, collagen deposition and phosphorylation.[17,22] In particular, there is evidence from experimental research studies suggesting that microbiota impacts AKI outcome.[12] Overall, in spite of the limited evidence in the specific pathophysiological mechanisms involving microbiota metabolites and by-products implicated in AKI, it is fair to assume there is a strong association between dysbiosis and AKI.

The MELD score has been used for years to prioritize and allocate patients awaiting to receive a liver transplant and have proven to be accurate in stratifying patients according to their survival risk short-term (3-months).[23] In spite of its success, it is clear that since patients with chronic liver disease awaiting liver transplantation present a complex clinical profile, there are conditions that the MELD score fails to account properly.[23] In our study, we were able to indicate that VRE colonization is a better predictor of clinical complications in the liver transplant than the MELD score. To emphasize, dysbiosis plays a major role in metabolism, immunity and physiology in chronic liver diseases.[24–27]An alteration of the microbiota composition may superimpose the reason VRE colonization as a better predictor for complications in the liver transplant, especially after 3-months post-transplantation for its important role in chronic liver diseases.

Finally, in spite of VRE colonization not presenting any difference in the liver transplant survival in our population, the risk for mortality in the VRE colonized might be higher because restauration of the microbiota dysregulation post-transplant is more complicated because of the antibiotic treatment exposure and cofounding complications. Slow restauration of the microbiota

diversity does not allow the microbiome to regain healthy functionality and production of beneficial by-products, which as mentioned are important for their role in homeostasis, immunity, inflammation and cell-signaling mechanisms.

5.3 STRENGTHS OF THE STUDY

Our research methods include multiple variables that affect the liver transplant outcome allowing us to examine and consider co-founding factors to analyze multiple outcomes. This type of retrospective cohort approach is useful to study VRE and liver transplant population as it is a specific cohort in the infectious diseases field. In addition, our database of liver transplant patients is the second largest one in Canada allowing us to include a sufficiently large sample size to conduct the study. The University of Alberta Hospital receives the largest number of liver transplant in Western Canada providing care and follow-up to a large cohort of liver transplant patients.

5.4 LIMITATIONS OF THE STUDY

The retrospective nature of our cohort study superimposes some limitations. We found that VRE colonization is associated with complications on the liver transplant and its presence increase the risk for survival, however, through our research methods and design of the study, we were not able to prove the presence of dysbiosis due to the VRE colonization. In order to demonstrate microbiota changes because of VRE colonization, a study gathering stool samples to analyze the microbiota of the VRE colonized versus non-VRE colonized liver transplant patient needs to be conducted to confirm causality. In addition, retrospective cohort studies present a challenge in having to control over the nature and quality of the data.

5.5 STUDY SIGNIFICANCE

Our study present significant findings related to association of VRE colonization with liver transplant complications and outcome. Previous studies have researched the associations of VRE infection with the liver and solid organ transplants (SOT) broadly. However, we can state that since in our study population the incidence of VRE infection was very low and of no impact in the health outcome post-transplant, the presence of VRE colonization itself as a marker of dysbiosis suggests management of VRE colonization should be improved in the liver transplant

population. VRE colonization is clearly associated with acute kidney injury and presents a tendency for higher risk of mortality. The presence of VRE colonization as a marker of gut microbiota dysbiosis would open the door for further research supporting the claim of the significance in having a functional and a healthy microbiota in the chronic liver disease patients undergoing transplantation. Our findings also encourage further research in the role of the microbiome in the solid organ transplant field to improve health outcomes.

5.6 CONCLUSION

VRE colonization pre-transplant was associated with acute kidney injury, higher risk for renal injury and a tendency for higher mortality risk. VRE colonization is an independent and better predictor of complications in the liver transplant than MELD. Our findings suggest that optimizing management of this patient population during the peri-transplant period should include renal-protective strategies in VRE+ patients. Moreover, as our study also states that VRE colonization may be a surrogate marker of dysbiosis in the liver transplant, results encourage further exploration of microbiota dysregulation and the presence of VRE colonization in the gut. In conclusion, VRE colonization has a significant association with complications on the liver transplant and with liver transplant patients' health outcomes.

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