Indian Journal of Gastroenterology (January–February 2022) 41(1):37–51
https://doi.org/10.1007/s12664-021-01157-9

ORIGINAL ARTICLE

The role of gut microbiota in clinical complications, disease severity, and treatment response in severe alcoholic hepatitis

Cyriac Abby Philips[1,2] · Philip Augustine[3] · Karthik Ganesan[4] · Shatakshi Ranade[5] · Varun Chopra[5] · Kunal Patil[5] · Sonie Shende[5] · Rizwan Ahamed[3] · Sandeep Kumbar[3] · Sasidharan Rajesh[6] · Tom George[6] · Meera Mohanan[7] · Narain Mohan[1] · Nikhil Phadke[5] · Mridula Rani[5] · Arjun Narayanan[8] · Suchetha M. Jagan[8]

Received: 4 April 2020 / Accepted: 1 February 2021 / Published online: 6 January 2022

Abstract

Background Dysbiotic gut bacteria engage in the development and progression of severe alcoholic hepatitis (SAH). We aimed to characterize bacterial communities associated with clinical events (CE), identify significant bacteria linked to CE, and define bacterial relationships associated with specific CE and outcomes at baseline and after treatment in SAH.

Methods We performed 16-s rRNA sequencing on stool samples (n=38) collected at admission and the last follow-up within 90 days in SAH patients (n=26; 12 corticosteroids; 14 granulocyte colony-stimulating factor, [G-CSF]). Validated pipelines were used to plot bacterial communities, profile functional metabolism, and identify significant taxa and functional metabolites. Conet/NetworkX® was utilized to identify significant non-random patterns of bacterial co-presence and mutual exclusion for clinical events.

Results All the patients were males with median discriminant function (DF) 64, Child–Turcotte–Pugh (CTP) 12, and model for end-stage liver disease (MELD) score 25.5. At admission, 27%, 42%, and 58% had acute kidney injury (AKI), hepatic encephalopathy (HE), and infections respectively; 38.5% died at end of follow-up. Specific bacterial families were associated with HE, sepsis, disease severity, and death. *Lachnobacterium* and *Catenibacterium* were associated with HE, and *Pediococcus* with death after steroid treatment. Change from *Enterococcus* (promotes AH) to *Barnesiella* (inhibits *E. faecium*) was significant after G-CSF. Phenylpropanoid-biosynthesis (innate-immunity) and glycerophospholipid-metabolism (cellular-integrity) pathways in those without infections and the death, respectively, were upregulated. Mutual interactions between *Enterococcus cecorum*, *Acinetobacter schindleri*, and *Mitsuokella* correlated with admission AKI.

Conclusions Specific gut microbiota, their interactions, and metabolites are associated with complications of SAH and treatment outcomes. Microbiota-based precision medicine as adjuvant treatment may be a new therapeutic area.

Keywords Alcohol · Cirrhosis · Dysbiosis · Fecal transplant · Intestinal bacteria · Metabolomics · Metagenomics · Microbiome · Portal hypertension

✉ Cyriac Abby Philips
abbyphilips@gmail.com

[1] The Liver Unit and Monarch Liver Lab, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682 028, India

[2] Philip Augustine Associates (P) Ltd, Ernakulam Medical Center, Room no: 3. Ground Floor, Kochi 682 028, India

[3] Gastroenterology and Advanced G.I. Endoscopy, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682 028, India

[4] Biomedical Software and Instrumentation, Department of Bioinformatics, Helicalbio, Ann Arbor, MI, USA

[5] Molecular, Cellular and Developmental Biology, Genepath-Dx, Pune 411 004, India

[6] Interventional Radiology, The Liver Unit and Gastroenterology, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682 028, India

[7] Anaesthesia and Critical Care, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682 028, India

[8] National Urban Health Mission, Ernakulam District Hospital, Kochi 682 011, India

Bullet points of the study highlights

What is already known?

- Detrimental changes in quality and diversity of gut microbiota (GM) is linked to cirrhosis and associated complications.

What is new in this study?

- Specific changes in gut bacterial diversity, genera, their metabolic function and interactions are associated with severe alcoholic hepatitis (SAH) related clinical events, overall outcome and treatment response.

What are the future clinical and research implications of the study findings?

- Precision-medicine based on metagenomic studies on GM to target deleterious or supplement beneficial genera or their metabolites may help to improve treatment outcomes in SAH.

Introduction

Alcoholic liver disease (ALD) is a leading cause of liver-related morbidity and mortality worldwide. It encompasses a spectrum ranging from simple alcoholic steatosis to cirrhosis and the highly catastrophic or severe alcoholic hepatitis (SAH) in patients with excessive alcohol use within 12 weeks that can present with acute decompensation or acute on chronic liver failure (ACLF) in patients with underlying chronic liver disease (CLD) [1]. Health and disease in humans are dependent on the status of microbiota, and conditions that promote an unhealthy change in the microbiota (the quantitative and qualitative change called dysbiosis) can impact a disease process by affecting either initiation or promotion of progression. Studies on gut microbiota through fecal sampling studies on animal models as well as humans have shed light on the presence of distinct changes in gut bacterial communities, their metabolic function, and specific disease entities in ALD [2]. Pilot studies in small animal models and humans have also demonstrated the role of microbial modulation through probiotics and healthy donor fecal microbiota transplantation (FMT) or ideally termed intestinal microbiota reinstitution therapy (IMRT) in improving dysbiosis that ultimately translates to beneficial clinical outcomes in the diseased host. Multiple studies have shown associations between a specific group of microbiota and their disruptive functional metabolism and clinical events in patients with cirrhosis [3]. For example, Llopis et al. showed that *Streptococci*, *Enterobacteria*, and *Bifidobacteria* were associated with SAH, *Enterobacteria* were associated with higher bilirubin levels, and *Clostridia* were associated with lower levels of jaundice in humanized germ-free mice. Grander and colleagues found that *Akkermensia muciniphilia* levels in fecal samples were associated with the severity of ALD [4, 5]. Specific associations or relationships concerning intestinal bacterial communities and clinical events and therapeutic responses in humans with SAH remain unstudied. Our current study aimed to identify specific bacterial communities that are significantly associated with liver disease severity and liver-related events in patients with alcoholic hepatitis (AH) and analyzed relationships between bacterial groups and specific clinical events utilizing integrated bioinformatics tools.

Study objectives

a. To characterize significant bacterial communities and functional metabolites with clinical events such as the severity of hyperbilirubinemia, infections, hepatic encephalopathy (HE), and acute kidney injury at admission and on follow-up at 90 days, hyponatremia at admission, and death after diagnosis, in patients with SAH
b. To distinguish and identify significant bacterial groups and associated functional metabolites in patients with SAH after corticosteroid or granulocyte colony-stimulating factor (G-CSF) therapy
c. To identify significant relationships between pertinent bacterial taxa associated with clinical events in SAH patients using network analysis

Methods

Patients

From January 2019 to December 2019, consecutive patients with definite SAH (diagnosed as per clinical, biochemical, and

histological criteria as defined by Singal et al.) presenting to the emergency department and intensive care unit of the liver unit and Gastroenterology Department were included [6]. The study was a retrospective analysis of prospectively collected data. The study and retrospective collection of data were approved by the institutional ethics committee and was performed following the ethical standards of the 1964 Declaration of Helsinki and its later amendments. SAH was treated either with corticosteroids or with G-CSF as per patient/family preference and informed consent. Informed consent was taken from the patient/next of kin for collection of stool samples for next-generation sequencing of microbiota at admission and at the last follow-up or at 12 weeks after treatment, whichever came first. Those willing for liver transplantation at the outset or during medical treatment were referred to a transplant center for definitive management and were excluded from this study. Those patients in whom consent for transjugular liver biopsy was not provided and those with a clinical and biochemical diagnosis of AH in whom a second etiology of liver injury was either confirmed or contemplated were also excluded. Examples include concomitant drug-induced liver injury due to known hepatotoxic prescription drug use or complementary and alternative medicines; use of known hepatotoxic agents; acute or chronic viral hepatitis or reactivation of viral hepatitis; the presence of positive autoimmune hepatitis antibodies; chronic cholestatic liver disease; Wilson's disease; hepatocellular carcinoma; or portal vein thrombosis. Those with extrahepatic malignancies undergoing interventional vascular hepatobiliary or non-hepatobiliary procedures, severe cardiopulmonary disease, and those with >3 organ failures on multiple organ support were also excluded. All patients with diagnosed SAH were started on intravenous third-generation cephalosporins, and antibiotics were modified as per sepsis diagnosis and drug sensitivity. All patients were on zinc supplementation and oral or rectal lactulose. All patients with HE received rifaximin during the hospital stay; this was discontinued on discharge from the medical facility. None of the patients underwent liver transplantation or other salvage therapies such as fecal microbiota transplantation during the follow-up period. The study flow chart is shown in Supplementary Fig. 1.

Sample collection and DNA extraction, sequencing of bacterial communities, and bioinformatics

Stool samples (preferably Bristol score 3 or 4) were collected within 24 h of hospital admission in sterile containers. The samples were immediately processed into aliquots within 1 h and stored at – 80 °C. Follow-up (completion of 12 weeks or last admission/death whichever came earlier) stool samples were collected at the medical facility or transported in sterile containers from home within 6 h of collection. When a minimum of ten samples was collected during a period, RNAlater™ (Ambion™/Invitrogen™, Thermo Fisher Scientific, MA, USA) was added to the aliquots and transported to the main laboratory facility within 4 h along with maintenance of cold-chain for DNA extraction and further analysis [7]. A published and validated methodology was utilized for next-generation sequencing including variable region of gene sequenced, in-house developed/validated primers, and quality control of Illumina outputs [8]. Briefly, approximately 200 mg of the provided stool sample was used for bacterial DNA extraction using a defined and published validated protocol modification of the commercially available QIAmp DNA Stool Mini Kit1 (Qiagen, Venlo, Netherlands). Sequencing was performed on an Illumina MiSeq next-generation sequencer (Illumina, CA, USA) using the Illumina kit V.3 at 2 × 300 paired-end sequencing and classified taxonomically according to the GreenGenes Database (version 13.8). Further aspects of the raw sequencing data quality such as per-base quality, per-base guanine-cytosine (GC) content, and sequence length distribution were performed using standardized Illumina™ Output Fastq format and quality control. Alpha diversity for species richness was ascertained using a Chao1 index measure since the study included homogenous diagnosis (AH) in which gut microbiota study was performed at the same site (stool). The Quantitative Insights Into Microbial Ecology (QIIME version 2) was used to ascertain the quantitative and qualitative microbial communities. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway was utilized to study functional metabolism interactions and relations within the sequenced bacterial communities.

CIRCOS® representation, statistical methods, and network analysis

We utilized the visualization tool called Circos® (v.0.69-9; tools version: 0.23) to facilitate exploration and analysis of similarities and differences arising from comparisons of bacterial communities between variables. The table viewer script in Circos®-tools was used to format the abundance data. This method of representation shows significant positive, negative, or neutral interactions between communities specified for the variable. Representation of output data was performed using a circular chord ideogram layout for display of relationships between microbial communities concerning clinical or investigational variables at the taxonomy levels (phylum/class/order/family). To simplify the significant Circos® plots findings, a representational plot image followed by grouped associations retrieved from the plots was presented in a tabular manner [9]. The Phylogenetic Investigation of Communities

by Reconstruction of Unobserved States (PICRUSt, v 1.1.1) was used for predictive metabolic functional profiling of microbial communities using 16S rRNA marker gene sequences (precalculated for protein-coding genes present in KEGG gene families and 16S rRNA gene copy number). The linear discriminant analysis effect size (LEfSe) combined with the Kruskal–Wallis and pairwise Wilcoxon tests were utilized to identify significantly different microbial communities in their abundance and functionality between groups. The Benjamini and Hochberg false discovery rate correction test was applied during multiple comparisons during biomarker discovery (taxa and functional metabolites) as well as during network analysis as a separate step. Bray–Curtis dissimilarity analysis represented by dendrograms on principal coordinate analysis was performed. *P*-values determined by permutational multivariate analysis of variance were utilized adjusting for potentially confounding factors. We used default significance (alpha value = 0.05) and linear discriminant analysis thresholds (2.0) at all taxonomic levels in patients between time points [10, 11]. Networks were inferred by CoNet® (v.1.1.1-beta) applications within Cytoscape™ (v.3.7.2). The following measures were implemented by CoNet® and used to detect co-presence/exclusion between bacterial communities: Pearson, Spearman, Mutual Information, Bray–Curtis dissimilarity, and Kullback–Leibler dissimilarity. For computing, the significance of the association between each pair of nodes and bootstrapping with the ReBoot feature of CoNet was used. Edges (associations) with a *p*-value of less than 0.05 were retained and considered significant. CoNet was set up to be aware of the "comparison metadata" for each sample (i.e. the respective clinical or investigational variables such as bilirubin level cut-off and absence/presence of infection, etc.). The following network measures were calculated by NetworkX® (Version: 2.2): degree centrality, betweenness centrality, and closeness centrality. These were all added to the node attributes in the graph. The centrality measure of *degree* assigns the importance of a specific node (microbe at bacterial phylum/family/genus level) in the form of the number of direct connections to other nodes (microbes). The centrality measure of *betweenness* represents the number of times a microbe lies on the shortest path between other microbes. This shows which microbes are the best "bridges" between other microbes in the network. This measure demonstrates the bacteria that influence flow around a system (the clinical or investigational variable in this case). A high betweenness indicates the microbe that holds authority over different clusters in the specified network. The centrality measure of *closeness* represents microbial (nodal) closeness to all other nodes (microbes) in the network. It gives us a measure of the shortest path between all nodes (microbes) and identifies the individual microbe best placed to influence the entire network more quickly or those who are good "broadcasters" of information (metabolic cross-talk). The topology of constructed networks and significant taxa (top 2% of all taxa analyzed) were represented in a simplified tabular form based on these attribute indices of degree, closeness, and betweenness centrality measures [12–15].

General statistical analysis

Statistical analysis was performed using MedCalc for Windows, version 19.4 (Medcalc Software, Ostend, Belgium) and Number Cruncher Statistical Systems (NCSS) Statistical Software (NCSS, LLC., Utah, USA). Data are presented as mean and standard deviation or as median, based on variable skewness/distribution or log transformation, as applicable. The Shapiro–Wilk test was used to test normality, Levene's test was used for non-nominal data, and the Bartlett homogeneity test for nominal variables was utilized to check for equality of variances. A logarithmic transformation was applied to decrease the variability of data and make data conform more closely to the normal distribution. Chi-square and Fisher's exact tests were used to compare nominal variables. A Mann–Whitney's *U* test was used to evaluate continuous variables. The probability of patients surviving up to the study endpoint was calculated using the Kaplan–Meier method as represented by the survival time curve. A comparison between the survival curves was made using the log-rank test, and p-values < 0.05 were considered significant.

Results

Patients and characteristics at baseline

A total of 26 patients with SAH were included in the study: 12 on corticosteroids and 14 on G-CSF. Next-generation sequencing was performed on a total of 38 stool samples collected at baseline and follow-up. All patients were males with a mean age of 47.3 ± 9.1 years. On admission, the median total bilirubin was 10 mg/dL, mean international normalized ratio was 2.3 ± 0.5, creatinine was 1.12 ± 0.6 mg/dL, and serum sodium was 129.6 ± 5.3 mmol/L. The median Child–Turcotte–Pugh (CTP), discriminant function (DF), and model for end-stage liver disease (MELD) scores at admission were 12, 64, and 25.5, respectively. Acute kidney injury, overt HE, and confirmed infections were noted in 73.1%, 57.7%, and 42.3% of cases on admission, respectively. CTP >10, DF >65, and MELD >25 were notable in 61.5%, 46.2%, and 50%, respectively. Out of 12 patients on CS, five (41.6%) had controlled infection at start of treatment, two developed infection during course of CS, which was continued after control of infection, and only two (16.7%) had AKI at admission, while five (41.6%) developed AKI on follow-up. A total of seven patients (58.3%) on CS developed infections at end of complete follow-up. Infections were notable in 10 patients (71.4%) at start of treatment in G-CSF group; total seven

(50%) developed infections during completion of follow-up. In G-CSF group, AKI was noted in five (35.7%) patients at start of therapy, while seven patients (50%) developed AKI at end of follow-up. The overall survival (n=26) was 73.7±4.6 days; 74.1±6.3 days in those receiving CS; and 73.3±6.7 days in those on G-CSF. Overall, 38.5% died at 3 months follow-up without any significant differences on Kaplan–Meier analysis on survival between treatment groups (corticosteroid 58.3% vs. G-CSF 64.3%, p=0.86).

Bacterial communities at baseline and between grouped variables

Bacterial diversity and qualitative bacterial communities and associated clinical variables

A total of 38 stool samples from 26 patients underwent next-generation sequencing yielding 3511 observations. The total count was 3,441,297 with minimum count of 8448, maximum of 131,797, median of 90,247, mean of 93,008.02, and standard deviation 24,366.35 on observed metadata categorization of taxonomy. The alpha diversity (within sample variance) between patients was demonstrated using the Chao1 index to judge the magnitude of the differences (using analysis of similarity, ANOSIM) in richness among communities organized according to baseline clinical variables as well as follow-up grouping (Supplementary Fig. 2). The alpha diversity within samples at baseline groups was not significantly different except for hyponatremia, AKI at admission, MELD score severity (negative correlation: higher diversity, lower severity), and those with HE on follow-up ($p < 0.05$). Using the QIIME (v.2) and GreenGenes microbial gene database, we noted that the relative abundances (RA) of bacteria at the phylum, family, and genus levels differed substantially for clinical variables at admission and on follow-up. Briefly, *Firmicutes* and *Proteobacteria* (represented by *Corynebacterium*, *Streptococcus*, *Megasphaera*, *Ruminococcus*, *Sutterella*, *Enterobacter*) were significantly higher in patients with overt HE at admission, while *Actinobacteria* and *Bacteroidetes* (consisting mainly of *Bifidobacterium*, *Collinsella*, *Slackia*, *Bacteroides*, *Lachnospira*, *Trabulsiella*) were relatively abundant in those without overt HE on follow-up. Similarly, *Corynebacterium*, *Bifidobacterium*, *Collinsella*, *Bacteroides*, *Streptococcus*, *Ruminococcus*, *Veillonella*, *Klebsiella*, and *Akkermansia* were the predominant genera associated with infection in SAH patients at admission. In contrast, RA of *Actinomyces*, *Corynebacterium*, *Coriobacteria*, *Atopobium*, *Prevotella*, *Dialister*, *Megamonas*, *Megasphaera*, and *Klebsiella* was associated with the occurrence of infection on follow-up at 90 days. Similarly, striking changes were notable between RAs of bacterial communities based on baseline hyperbilirubinemia cut-off (≥ 10 mg/dL), presence or absence of overt HE at admission and follow-up, hyponatremia, and AKI on admission and follow-up (Supplementary Tables 1 and 2). Importantly, species richness/diversity at baseline and significant specific bacterial taxa association were notable among patients of SAH with hyponatremia, renal injury, and disease severity (MELD score) at admission and those developing HE on follow-up. Using the Circos™ graphical analysis, key bacterial communities up to the *family level* were identified and associated with specific clinical variables (Fig. 1). Interestingly, bacterial families associated with degradation of aromatic compounds (*Rhodocyclaceae*), tolerant to heavy-metal stress (*Exiguobacteraceae*), denitrification (*Caulobacteraceae*), and entero-pathogenic (*Enterobacteriaceae*, *Enterococcaceae*), were abundant in patients with HE at presentation. In contrast, those associated with nitrogen fixation (*Oxalobacteraceae*); methane metabolism (*Victivallaceae*); non-pathogenic, vegetative, and highly radiation-resistant (*Deinococcaceae*); and commensal (*Dermabacteraceae*) bacteria were specifically associated with the absence of HE at admission. Similarly, bacterial families associated with various resistance patterns to antimicrobials (*Veillonellaceae*), those harboring opportunistic pathogens of humans associated with periodontal disease (*Prevotellaceae*), and those associated with ancient intrinsic resistome profiles resulting in antibiotic ineffectiveness (*Paenibacillaceae*) were specifically abundant in SAH patients with infections on admission. *Turicibacteraceae* was specifically associated with lower IgA coating of gut mucosa and was associated with higher DF, CTP, and MELD scores in patients with SAH. Details of all bacterial communities associated with specific clinical variables in SAH patients on Circos® analysis are shown in Supplementary Table 3.

Significant bacterial communities and functional metabolism associated with clinical variables

After identification of the bacterial population concerning RAs and key bacterial families associated with specific clinical events and liver disease severity, the LEfSe method was used to determine significant bacteria and respective metabolic functions most likely to explain differences between grouped clinical and investigational variables. This method coupled standard tests for statistical significance with additional tests encoding biological consistency and effect relevance (Fig. 2). On LEfSe analysis, no significant difference was noted between the CS and G-CSF groups at baseline concerning bacterial communities and their functions. In patients without HE at admission, orally predominant bacteria such as *Odoribacter* (increases tryptophan metabolism and levels of favorable monoamine neurotransmitters), non-pathogenic commensal (*Actinobacillus*), and beneficial short-chain fatty acid (SCFA)–producing *Catenibacterium*

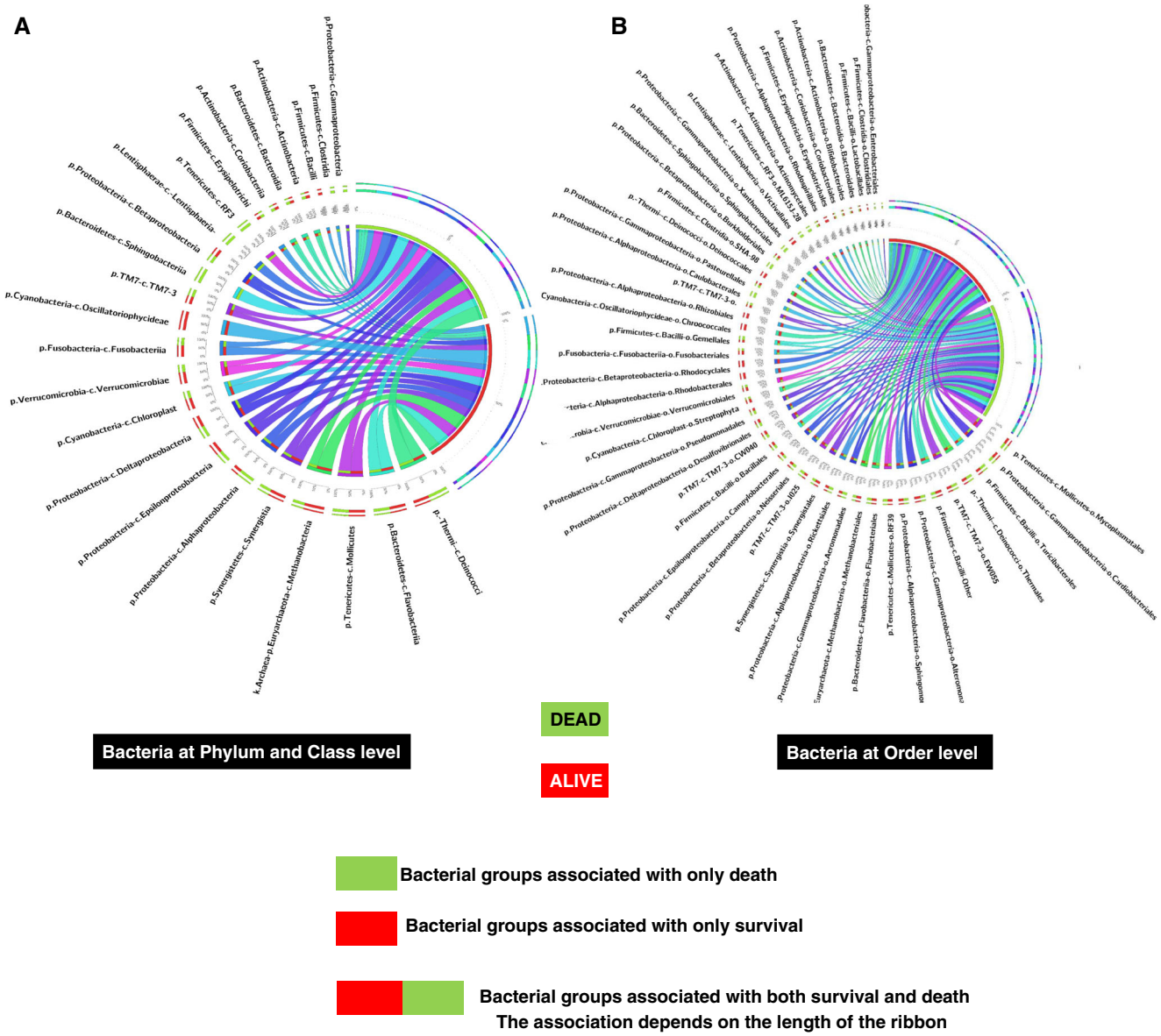

Fig. 1 Circos® plot representing bacterial taxa associated with overall outcomes (survival or death) among patients with severe alcoholic hepatitis. The plot illustrates significant associations between outcome (survival in red, death in green) and fecal bacterial taxa at admission. Taxa are scaled and listed by relative abundances represented by the several multi-colored ribbons. The relative abundances are illustrated by the sizes of each color segment in the inner circle, while the percentages outside segments refer to the contribution from each abundant taxon. A represents pertinent bacterial taxa at the phylum and class level, while B represents taxa at the order level. A simplified version of all of the Circos® output is shown in Supplementary Table 3

were significantly elevated. In contrast, in those with overt HE at presentation, pathogenic oral and respiratory mucosa-predominant genera (*Parvimonas*, *Selenomonas*, *Leptotrichia*), genera associated with antimicrobial resistance (*Peptostreptococcus*, *Mogibacterium*), and those with potent lipopolysaccharide (LPS, *Fusobacterium*) levels were notable. Pathways associated with ammonia scavenging (arginine and proline metabolism), the formation of secondary metabolites with anti-oxidant and anti-inflammatory activity (phenylpropanoid biosynthesis), and endogenous production of bacterial secondary metabolites with antimicrobial activity against many Gram-positive and some Gram-negative bacteria (ansamycin biosynthetic pathway) were significantly upregulated in patients without overt HE at baseline. Toxic and detrimental pathways such as degradation of nitroaromatics, C5-branched dibasic acid metabolism, and LPS synthesis were upregulated in SAH patients with overt HE at baseline. On follow-up at 90 days, SAH patients without infections (irrespective of treatment received) had strikingly significant bacterial genera associated with endogenous carbapenem production (*Erwinia*), genera that assist in bacterial competition,

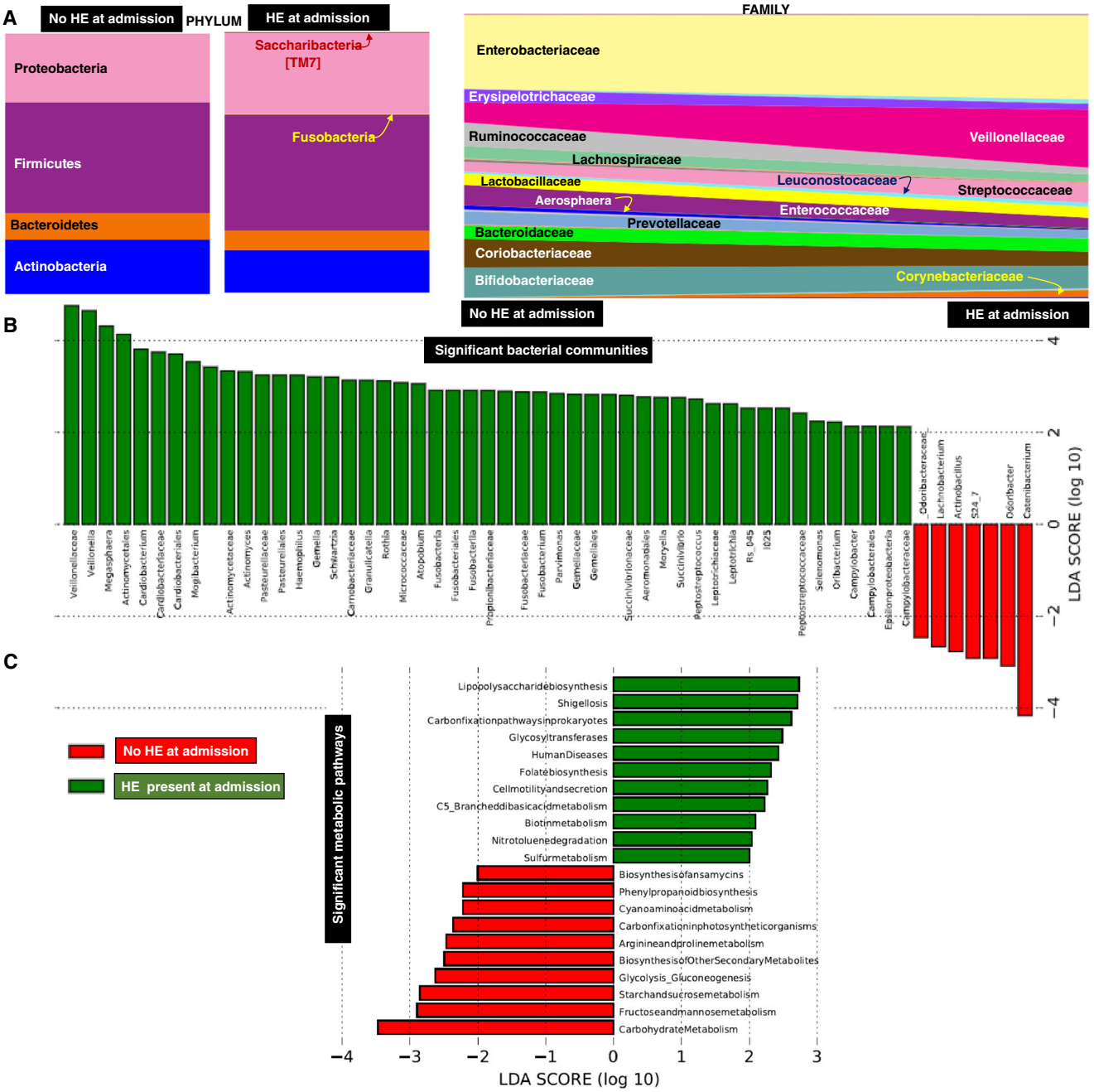

Fig. 2 (A) QIIME® based bar and area plots of relative abundances of bacterial taxa in patients with and without overt hepatic encephalopathy (HE) at admission in severe alcoholic hepatitis; (B) multivariate analysis using linear discriminant analysis effect size to identify significant taxa associated with and without HE at admission; (C) linear discriminant analysis effect size on functional metagenome utilizing Phylogenetic Investigation of Communities by Reconstruction of Unobserved States showing significant gut bacterial metabolic pathways associated with (green) and without (red) HE at admission in patients with severe AH

and genera that promote resistance to colonization in the host (*Trabulsiella*).

The significant abundance of the Actinobacterium *Eggerthella* associated with systemic bacteremia, drug inactivation, and immune dysregulation was remarkable in patients with a CTP score >10. This score was also associated with gut dysbiotic stress in the form of an upregulated functional pathway of vitamins and co-factor metabolism. In SAH patients with MELD score < 25, the Flavobacteria *Capnocytophaga*—an oral commensal of humans and canines with anti-LPS activity—was significantly upregulated. Similarly, the genus *Lautropia* with predominant oral-mucosa representation and pathogenicity in the immunosuppressed state was significant in the gut in SAH patients with hyponatremia. In patients receiving CS, a significant abundance in *Prevotella* and

Table 1 Significant bacterial taxa between clinical, investigational, disease severity, treatment outcome and overall outcomes grouped in patients with severe alcoholic hepatitis

Presence of hepatic encephalopathy at admission	**Absence of hepatic encephalopathy at admission**
Significant bacterial genera	
Veillonella, Megasphaera, Cardiobacterium, Mogibacterium, Haemophilus, Gemella, Schwartzia, Granulicatella, Rothia, Atopobium, Fusobacterium, Parvimonas, Succinivibrio, Peptostreptococcus, Leptotrichia, Moryella, Selenomonas, Oribacterium, Campylobacter	Odoribacter, Actinobacillus, Catenibacterium
Significant functional metabolism	
Lipopolysaccharide biosynthesis	Biosynthesis of ansamycins
Carbon fixation pathways	Fructose and mannose metabolism
Glycosyltransferases	Starch and sucrose metabolism
Folate biosynthesis	Biosynthesis of other secondary metabolites
C5-branched dibasic acid metabolism	Arginine and proline metabolism
Biotin metabolism	Cyanoamino acid metabolism
Nitrotoluene degradation	Phenylpropanoid biosynthesis
Sulfur metabolism	
Presence of hepatic encephalopathy on follow-up	**Absence of hepatic encephalopathy on follow-up**
Significant bacterial genera	
Delftia	Acidaminococcus
Pediococcus	Trabulsiella
	Bifidobacterium
Significant functional metabolism	
No significant pathway(s) identified	Phenylpropanoid biosynthesis
Presence of infections at presentation	**Absence of infections at presentation**
Significant bacterial genera	
Schwartzia	Pseudoramibacter/Eubacterium
Porphyromonas	Catenibacterium
Peptostreptococcus	Megamonas
Eikenella	
Oribacterium	
Presence of infections on follow-up	**Absence of infections on follow-up**
Significant bacterial genera	
No significant taxa identified	Erwinia
	Trabulsiella
	Acinetobacter
Significant functional metabolism	
No significant pathway(s) identified	Phenylpropanoid biosynthesis
	Cyanoaminoacid metabolism
	Bile secretion
Presence of acute kidney injury at presentation	**Absence of acute kidney injury at presentation**
Significant bacterial genera	
Eikenella	Escherichia
Leptotrichia	Sutterella
Schwartzia	Bulleidia
Leuconostoc	Enterococcus
Presence of acute kidney injury on follow-up	**Absence of acute kidney injury on follow-up**
Significant bacterial genera	
Eggerthella	Bifidobacterium
Kocuria	Erwinia
	Citrobacter
	Trabulsiella
Child–Turcotte–Pugh score ≤10 at admission	**Child–Turcotte–Pugh score >10 at admission**

Table 1 (continued)

Presence of hepatic encephalopathy at admission	Absence of hepatic encephalopathy at admission
Significant bacterial genera	
Eubacterium	Eggerthella
Significant functional metabolism	
Hypertrophic cardiomyopathy pathway Cyanoamino acid metabolism	Metabolism of co-factors and vitamins
MELD score < 25 at admission	**MELD score ≥ 25 at admission**
Significant bacterial genera	
Capnocytophaga	Bacteroides Ruminococcus Clostridia Lachnospira
Significant functional metabolism	
Glycan degradation Sphingolipid metabolism Streptomycin biosynthesis	Signal transduction mechanisms
Discriminant function ≤65 at admission	**Discriminant function > 65 at admission**
Significant bacterial genera	
No significant taxa identified	Propionibacteria Gemella Fusobacteria
Significant functional metabolism	
Membrane transporters	No significant pathway(s) identified
Total bilirubin ≤10 mg/dL at admission	**Total bilirubin > 10 mg/dL at admission**
Significant bacterial genera	
Aerococcus Parabacteroides Collinsella	Actinomyces Granulicatella Porphyromonas Lautropia Oribacterium Neisseria
Hyponatremia (serum sodium < 130 mmol/L) at admission	**Hyponatremia (serum sodium >130 mmol/L) at admission**
Significant bacterial genera	
Lautropia	Prevotella
Before corticosteroid therapy (baseline)	**After corticosteroid therapy**
Significant bacterial genera	
Fusobacterium Rothia Micrococcus Dorea Blautia	Enhydrobacter Pediococcus
Significant functional metabolism	
No significant pathway(s) identified	Sulfur metabolism Glycosaminoglycan degradation Taurine and hypotaurine metabolism
Before G-CSF therapy (baseline)	**After G-CSF therapy**
Significant bacterial genera	
Streptophyta S Enterococcus	Barnesiella
Significant functional metabolism	
Styrene degradation	No significant taxa identified
Corticosteroid-treated patients who survived	**Corticosteroid-treated patients who died**

Table 1 (continued)

Presence of hepatic encephalopathy at admission	Absence of hepatic encephalopathy at admission
Significant bacterial genera	
Prevotella	Pediococcus
G-CSF-treated patients who survived	**G-CSF-treated patients who died**
Significant bacterial genera	
No significant taxa identified	Streptococcus Neisseria Granulicatella Flavobacteria TG-5
Significant functional metabolism	
No significant pathway(s) identified	RNA transport pathway
Overall survived at 3 months (baseline)	**Overall died at 3 months (baseline)**
Significant bacterial genera	
Bifidobacterium	Aerococcus
Significant functional metabolism	
No significant pathway(s) identified	Glycerophospholipid metabolism

The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, v 1.1.1) was used for predictive metabolic functional profiling of microbial communities using 16S rRNA marker gene sequences (precalculated for protein-coding genes present in KEGG gene families and 16S rRNA gene copy number). The linear discriminant analysis effect size (LEfSe) combined with the Kruskal–Wallis and pairwise Wilcoxon tests was utilized to identify significantly different microbial communities in their abundance and functionality between groups. We used default significance (alpha value = 0.05) and linear discriminant analysis thresholds (2.0), at all taxonomic levels in the patients between time points. This table is a simplified form of the graphical output from multivariate bioinformatic pipelines utilized to identify significant bacterial taxa associated with specific clinical events. *MELD* model for end-stage liver disease, *G-CSF* granulocyte colony-stimulating factor

Pediococcus was associated with survival and death, respectively. In patients receiving G-CSF therapy at baseline, the *Enterococcus* genus that is known to be associated with SAH was preferentially modified toward an abundance of *Barnesiella*. *Barnesiella* can inhibit and clear vancomycin-resistant *Enterococci* naturally. The complete details of multivariate graphical output using LEfSe on significant bacterial communities, their functional metabolites, and associated clinical events are simplified and shown in Table 1.

Network analysis and bacterial communities' interaction associated with clinical variables

Network analysis for interactions using NetworkX™ revealed striking differences and interactions between bacterial communities based on clinical and investigational grouping (Fig. 3). For example, in patients with HE at admission, the genus *Granulicatella* was the most interactive even though the most abundant was *Enterobacter*. Concurrently, *Peptostreptococcus* was most influential and *Atopobium* was the genus with the highest level of metabolic cross-talk with other genera. Similarly, in SAH patients with CTP >10, the genus *Clostridia* was the most abundant and *Granulicatella* had the most interactions and metabolic cross-talk, while *Bulleidia* was the most influential. Complete details on interactions based on different groupings are shown in Supplementary Table 4. Using CoNet®, significant bacterial interactions were notable on grouping based on HE on follow-up at 90 days, infections at admission, and AKI at admission and follow-up. For example, a negative correlation between *Bacteroides*, *Parabacteroides*, *Erwinia*, and *Citrobacter* genera was significant in patients with HE at follow-up. A higher abundance of *Enterococcus*, *Coprococcus*, *Megamonas*, and *Catenibacterium* was associated with a lower incidence of sepsis at admission in SAH patients (Fig. 4).

Discussion

Changes in gut bacterial communities are associated with cirrhosis and related clinical events. There is a paucity of data on distinct changes in the fecal microbiota in definite alcoholic hepatitis and related clinical events. In general, alpha and beta diversity-based analyses are utilized for community profiling. Our objectives were to utilize bioinformatic tools for comparative analysis for the study and identification of shared and

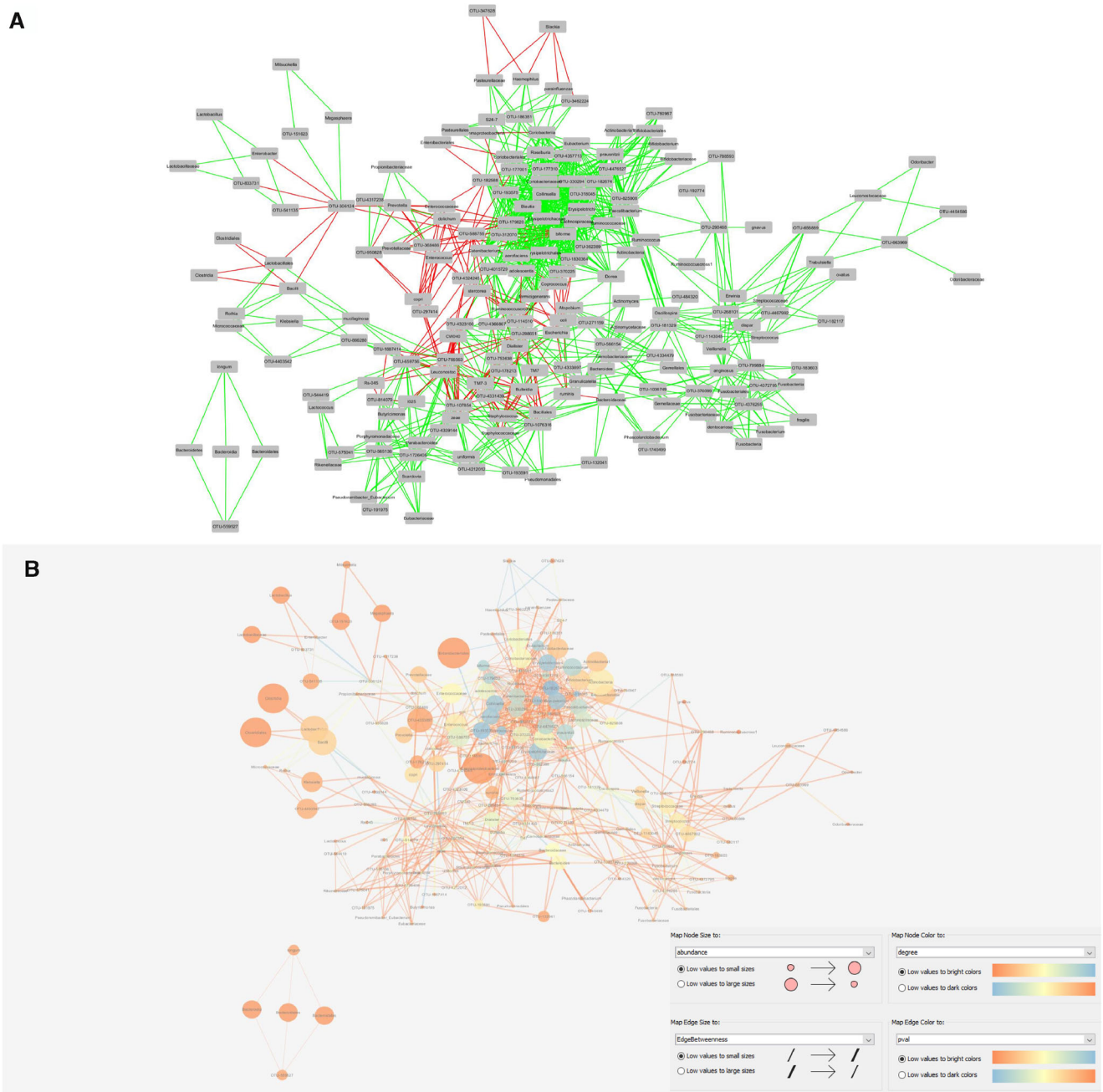

Fig. 3 A network analysis of bacterial community interactions in severe alcoholic hepatitis patients without infections at baseline visualized on Cytoscape® and inferred using NetworkX™. Panel A shows the positive (green) or negative (red) correlations between different bacterial family/genera (node name) concerning the absence of infections at admission. Panel B shows analysis as per NetworkX™ to appreciate measures of centrality between bacterial communities. The mapping attributes are shown in the inset at the bottom right corner of panel B. Panel B shows that the most abundant bacterial family/genera are not always the most influential or dominant genera concerning the clinical variable in the study.

unique bacterial taxa within samples belonging to the same cohort. In our study, we demonstrate that specific intestinal bacterial family/genera were associated with the disease severity, liver, and PHT-related clinical events at admission and follow-up as well as therapy-related outcomes in patients with SAH. We found that admission hyponatremia and kidney injury, encephalopathy on follow-up, and MELD score (negative correlation) at baseline were associated with both changes in alpha diversity (species richness) as well as significant taxonomical changes.

A change from autochthonous (indigenous) bacteria toward pathogenic genera has been shown in multiple studies in patients with cirrhosis. In the study by Chen et al. in patients with cirrhosis, *Streptococcaceae* correlated positively, while

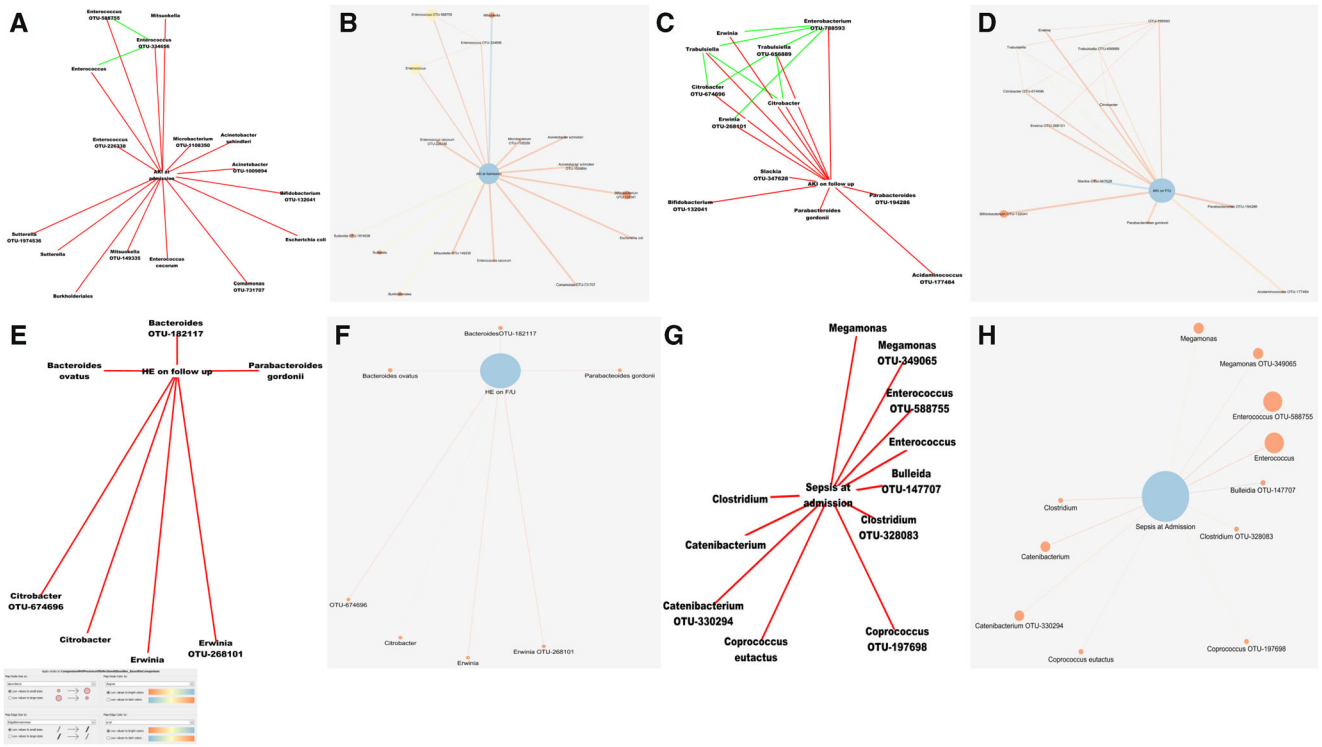

Fig. 4 Analysis outcomes of significant bacterial interactions concerning specific clinical variables in patients with severe alcoholic hepatitis (SAH). The variables have shown retained significance concerning associations (network edges) on Pearson, Spearman, and Bray–Curtis dissimilarity with $p < 0.05$. (A) Type of interaction (positive or negative/co-presence or mutual exclusion) between bacterial taxa concerning acute kidney injury (AKI) at admission. In contrast, (B) depth of the interaction concerning measures of centrality (degree, betweenness, and closeness) between significant bacterial taxa; C and D demonstrate AKI on follow-up; (E and F) hepatic encephalopathy on follow-up; and (G and H) sepsis at admission in patients with SAH

Lachnospiraceae correlated negatively with CTP severity [16]. Bajaj and colleagues found that *Veillonellaceae* was increased in cirrhotics with overt HE, while *Enterobacteriaceae* and *Ruminococcaceae* correlated positively and negatively with MELD scores, respectively. *Enterobacteriaceae* and *Veillonellaceae* were associated positively, and *Ruminococcaceae* correlated negatively with inflammation and endotoxemia. Bajaj et al. also found that several oral-origin bacterial genera were only found in decompensated cirrhosis. In yet another study, Roseburia was protective toward hospitalizations, while Enterococcus was associated with frequent hospitalizations in patients with decompensated cirrhosis. *Enterococcus*, *Megasphaera*, and *Burkholderia* were linked to poor cognition and inflammation in alcoholic cirrhosis [17]. In ACLF, Chen et al. found that a lower abundance of *Lachnospiraceae* was associated with overt HE, while another study showed that Proteobacteria (*Enterobacteriaceae*, *Campylobacteriaceae*, *Pasteurellaceae*) and Firmicutes (*Enterococcaceae* and *Streptococcaceae*) were associated with ACLF, death, and fast unit transfer [18, 19].

Zhang and co-workers showed that *Prevotellaceae* independently predicted 28-day mortality in patients with ACLF. In a recent study, Smirnova and colleagues studied the fecal microbiome in patients with heavy alcohol use and those with moderate and SAH [20]. These authors found that, similar to our observations, the development of AH was associated with distinct changes in the fecal microbiome. An increase in *Actinomycetaceae* and *Fusobacteriaceae* was noted among those with SAH. They also found that treatment with steroid or pentoxifylline altered gut microbiota differentially, but the microbiome composition did not distinguish between moderate and SAH based on MELD cut-offs. However, this study utilized basic methods of microbiota analysis in a very small group of patients and only at the "family" level without consideration for network analysis; thus, significant conclusions could not be made. Another study by Lang and colleagues demonstrated distinct changes in the gut bacterial microbiome of AH patients with more severe disease. The authors observed a negative correlation between the MELD score and bacterial diversity (which was also notable in our study). Subjects with SAH had significantly decreased RAs of *Akkermansia*, while that of *Veillonella* was increased; patients receiving steroids had an increase in *Veillonella* abundance [21]. Similar to their study, we found that Neisseria was associated with high bilirubin levels and MELD scores.

We further demonstrate that pathogenic oral and respiratory mucosa predominant genera with inherent antimicrobial

resistance (*Oribacterium*, *Rothia*, *Gemella*, *Peptostreptococcus*) and with potent LPS secretion (*Hemophilus*) were significant in patients with overt HE at admission, while on follow-up intestinal crypt-specific core genera that modulate epithelial cell proliferation and repression through potent LPS pathways (*Delftia*) were significant in those developing HE [22, 23]. In patients without infections at admission, genera associated with high resistance to oxidative stress and health-promoting commensal groups with oxalate degradation ability predominated (*Eubacterium*, *Catenibacterium*). On follow-up, quorum sensing and endogenous carbapenem-producing genera were significantly abundant in patients who did not develop sepsis (*Erwinia*) [24–28]. Similarly, enrichment of proinflammatory oral pathogens, aromatic oxidant stress-promoting genera, and bacteria notable for pathogenicity in immunosuppressed states (*Eikinella*, *Leuconostoc*, *Eggerthella*) were significant in patients with renal injury at admission and follow-up [29, 30].

We also found that the overall survival was dependent on the significant abundance of Bifidobacterium genera, while pathogenic *Aerococcus* genera and inflammation-associated pathways of glycerophospholipid metabolism predominated in those who died. Bifidobacterium species and their metabolic functions have been well described in the literature to promote gut health, improve immune functions, and substantiate beneficial commensal activity in the host [31–34]. Interestingly, we found that *Enterococcus* was associated with SAH at baseline following current literature; treatment with G-CSF resulted in preferential and natural inhibition of *Enterococcus* by *Barnesiella* genera [35, 36]. Multivariate network analysis showed that abundance was not the most important aspect of intestinal microbiota affecting clinical events in AH patients. This is underscored by the fact that identification of genera with lower abundance but with higher centrality measures of influence and dominance over other bacterial species was significantly associated with clinical and investigational variables. Our findings identify and support the possible role of precision medicine using targeted bacterial genera or their metabolites as synergistic therapy in patients with SAH for whom currently no approved treatments exist. A summary of the pertinent findings is shown in Fig. 5.

Intra- and inter-individual variability and diversity in the form of alpha and beta diversity is utilized when studying two different sets of samples at the baseline. Specifically, a high alpha diversity (the number of species in relation to the species' abundance within a sample) is linked to a healthy state and is utilized in health and disease or disease-severity comparisons. In our study, we applied various bioinformatic pipelines in a singularly affected patient group of SAH to identify microbiota and functionality associated with specific clinical

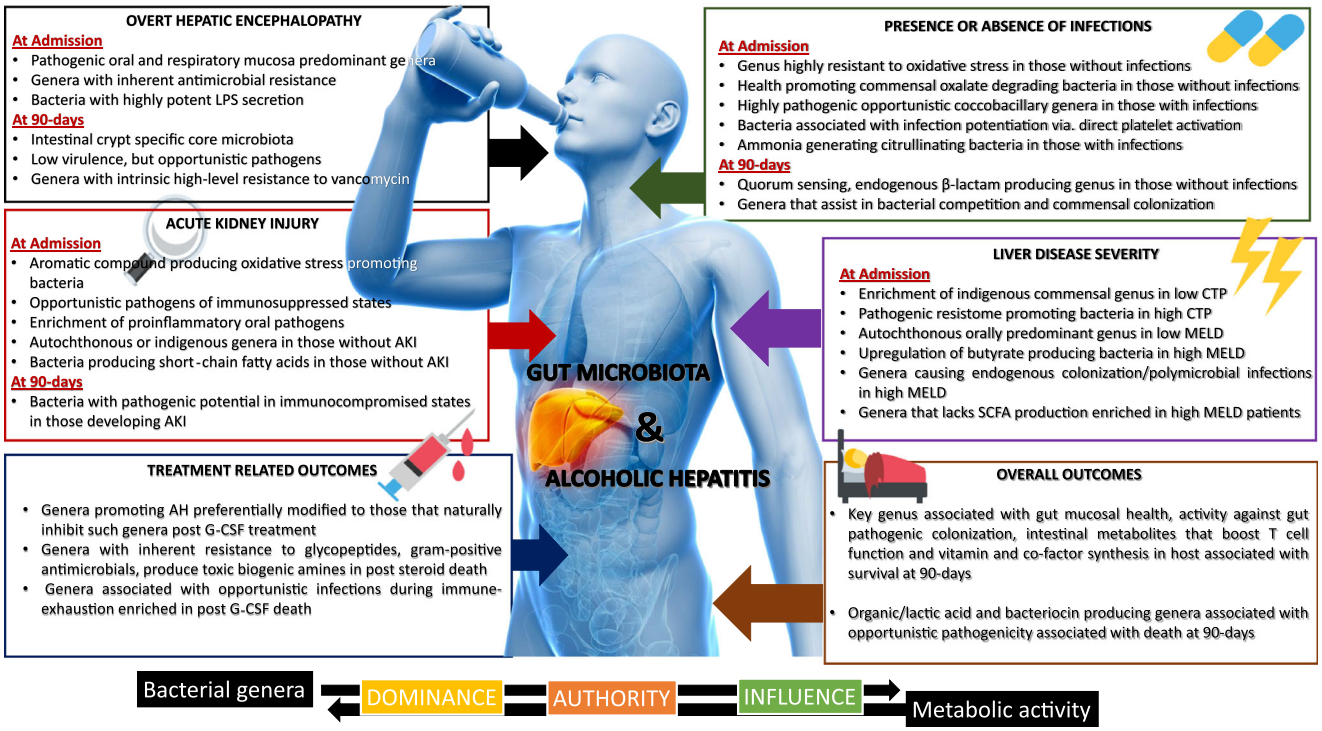

Fig. 5 Infographic summary of pertinent findings of the study
Human figure image component courtesy - @yooseohyun366 from imgbin.com
LPS lipopolysaccharide, *AKI* acute kidney injury, *G-CSF* granulocyte colony-stimulating factor, *CTP* Child -Turcotte -Pugh, *MELD* model for end-stage liver disease, *SCFA* short-chain fatty acid

events. We found that species diversity and taxonomic changes significantly correlated with only hyponatremia, kidney injury, and MELD score on admission and HE on follow-up.

Even though these studies were performed with strict inclusion criteria as well as systematic protocols for identification and analysis of fecal microbiota, our conclusions need further confirmation from other research groups and in a larger group of patients. A small proportion of patients with overt HE was treated with rifaximin during the admission period. This could have affected microbial communities during hospital admission. However, the use of rifaximin only briefly modulated the gut microbial diversity without a major change in the overall gut microbiota composition. This did not affect systemic inflammation or abnormalities of gut mucosal integrity such as the leaky gut [37, 38]. Even though patients did not receive antibiotics prior to baseline stool sample collection, several of them were on antibiotics during subsequent sampling. Several studies have shown that 7–14 days of antibiotics could result in significant dysbiosis that may persist over the long-term. Some studies also demonstrated that the gut bacteria recover after that period with some non-desirable bacterial species colonizing the gut [39, 40]. In our study, the second sampling was done after 3 months from the baseline, which could have affected dysbiosis and clinical outcomes in the short-term but did not affect current study clinical endpoints other than mortality. We did not consider the impact of the environment, dietary changes, use of beta-blockers, and nutritional supplements on the intestinal microbiota of our patients because such confounders were difficult to assess due to regional and dietary differences in our patient population. However, strict measures were taken for dietary measures in our cohort and were presumed to have been followed as per protocol.

In conclusion, our study on fecal microbiota in SAH patients demonstrates a specific role of significant bacterial genera and their functional metabolites affecting clinical and therapeutic outcomes and liver disease severity at presentation. Precision medicine utilizing beneficial genera or their metabolites as an adjunct therapy to improve clinical outcomes or specific clinical events in patients with SAH could become an important addition to the treatment armamentarium especially in this difficult-to-treat patient population for whom no approved treatment options exist.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12664-021-01157-9.

Author contribution CAP, PA, SR, and RA designed the study and wrote the manuscript; SK, RA, TG, MM, AN, and SMJ collected and collated data; KG, SS, KP, VC, SR, NP, and MR processed samples, extracted raw data, and performed bench analysis and bioinformatics; PA, KG, SR, RA, MM, TG, NP, MR, AN, and SMJ made critical revisions to the manuscript; all authors agreed to the final version of manuscript for submission

Compliance with ethical standards

Conflict of interest CPA, PA, KG, SR, VC, KP, SS, RA, SK, SR, TG, MM, NM, NP, MR, AN, and SMJ declare no competing interests.

Ethics statement The study was performed conforming to the Helsinki declaration of 1975, as revised in 2000 and 2008 concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

Disclaimer The authors are solely responsible for the data and the contents of the paper. In no way, the Honorary Editor-in-Chief, Editorial Board Members, the Indian Society of Gastroenterology or the printer/publishers are responsible for the results/findings and content of this article.

References

1. Seitz HK, Bataller R, Cortez-Pinto H, et al. Alcoholic liver disease. Nat Rev Dis Primers. 2018;4:16.
2. Philips CA, Augustine P, Yerol PK, et al. Modulating the intestinal microbiota: therapeutic opportunities in liver disease. J Clin Transl Hepatol. 2020;8:87–99.
3. Philips CA, Augustine P, Yerol PK, Rajesh S, Mahadevan P. Severe alcoholic hepatitis: current perspectives. Hepat Med. 2019;11:97–108.
4. Llopis M, Cassard AM, Wrzosek L, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Gut. 2016;65:830–9.
5. Grander C, Adolph TE, Wieser V, et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut. 2018;67:891–901.
6. Singal AK, Louvet A, Shah VH, Kamath PS. Grand rounds: alcoholic hepatitis. J Hepatol. 2018;69:534–43.
7. Wu WK, Chen CC, Panyod S, et al. Optimization of fecal sample processing for microbiome study - the journey from bathroom to bench. J Formos Med Assoc. 2019;118:545–55.
8. Philips CA, Phadke N, Ganesan K, Ranade S, Augustine P. Corticosteroids, nutrition, pentoxifylline, or fecal microbiota transplantation for severe alcoholic hepatitis. Indian J Gastroenterol. 2018;37:215–25.
9. Krzywinski M, Schein J, Birol I, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19:1639–45.
10. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31:814–21.
11. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.
12. Lotia S, Montojo J, Dong Y, Bader GD, Pico AR. Cytoscape app store. Bioinformatics. 2013;29:1350–1.
13. Faust K, Raes J. CoNet app: inference of biological association networks using Cytoscape. F1000Res. 2016;5:1519.
14. Faust K, Sathirapongsasuti JF, Izard J, et al. Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol. 2012;8:e1002606.
15. Hagberg AA, Schult DA, Swart PJ. Exploring network structure, dynamics, and function using NetworkX, in Proceedings of the 7th

Python in Science Conference (SciPy2008), Gäel Varoquaux, Travis Vaught, and Jarrod Millman (Eds), (Pasadena, CA USA), pp. 11–15, Aug 2008.
16. Chen Y, Yang F, Lu H, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology. 2011;54: 562–72.
17. Bajaj JS. Alcohol, liver disease and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16:235–46.
18. Chen Y, Guo J, Qian G, et al. Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality. J Gastroenterol Hepatol. 2015;30:1429–37.
19. Trebicka J, Bork P, Krag A, Arumugam M. Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure. Nat Rev Gastroenterol Hepatol. 2021;18:167–80.
20. Smirnova E, Puri P, Muthiah MD, et al. Fecal microbiome distinguishes alcohol consumption from alcoholic hepatitis but does not discriminate disease severity. Hepatology. 2020;72:271–86.
21. Lang S, Fairfied B, Gao B, et al. Changes in the fecal bacterial microbiota associated with disease severity in alcoholic hepatitis patients. Gut Microbes. 2020;12:1785251.
22. Garcia-Carretero R, Lopez-Lomba M, Carrasco-Fernandez B, Duran-Valle MT. Clinical features and outcomes of fusobacterium species infections in a ten-year follow-up. J Crit Care Med (Targu Mures). 2017;3:141–7.
23. Poyer F, Friesenbichler W, Hutter C, et al. Rothia mucilaginosa bacteremia: a 10-year experience of a pediatric tertiary care cancer center. Pediatr Blood Cancer. 2019;66:e27691.
24. Eribe ERK, Olsen I. Leptotrichia species in human infections II. J Oral Microbiol. 2017;9:1368848.
25. Naito T, Mulet C, De Castro C, et al. Lipopolysaccharide from crypt-specific core microbiota modulates the colonic epithelial proliferation-to-differentiation balance. mBio. 2017;8:e01680–17.
26. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009;294:1–8.
27. McGowan S, Sebaihia M, Jones S, et al. Carbapenem antibiotic production in Erwinia carotovora is regulated by CarR, a homologue of the LuxR transcriptional activator. Microbiology. 1995;141:541–50.
28. Kageyama A, Benno Y. Catenibacterium mitsuokai gen. nov., sp. nov., a gram-positive anaerobic bacterium isolated from human faeces. Int J Syst Evol Microbiol. 2000;50:1595–9.
29. Hayashi C, Gudino CV, Gibson FC 3rd, Genco CA. Review: Pathogen-induced inflammation at sites distant from oral infection: bacterial persistence and induction of cell-specific innate immune inflammatory pathways. Mol Oral Microbiol. 2010;25:305–16.
30. Gosset G. Production of aromatic compounds in bacteria. Curr Opin Biotechnol. 2009;20:651–8.
31. Azad MAK, Sarker M, Li T, Yin J. Probiotic species in the modulation of gut microbiota: an overview. Biomed Res Int. 2018;2018: 9478630.
32. Bubnov RV, Babenko LP, Lazarenko LM, Mokrozub VV, Spivak MY. Specific properties of probiotic strains: relevance and benefits for the host. EPMA J. 2018;9:205–23.
33. Arboleya S, Watkins C, Stanton C, Ross RP. Gut Bifidobacteria populations in human health and aging. Front Microbiol. 2016;7: 1204.
34. Zhang C, Wang K, Yang L, et al. Lipid metabolism in inflammation-related diseases. Analyst. 2018;143:4526–36.
35. Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature. 2019;575:505–11.
36. Ubeda C, Bucci V, Caballero S, et al. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. Infect Immun. 2013;81:965–73.
37. Jørgensen SF, Macpherson ME, Bjørnetrø T, Pompili M, Gasbarrini A. Rifaximin alters gut microbiota profile but does not affect systemic inflammation - a randomized controlled trial in common variable immunodeficiency. Sci Rep. 2019;9:167.
38. Ponziani FR, Zocco MA, D'Aversa F, Pompili M, Gasbarrini A. Eubiotic properties of rifaximin: Disruption of the traditional concepts in gut microbiota modulation. World J Gastroenterol. 2017;23:4491–9.
39. Francino MP. Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. Front Microbiol. 2016;6:1543.
40. Palleja A, Mikkelsen KH, Forslund SK, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol. 2018;3:1255–65.