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Longitudinal dysbiosis of gut bacteriome and mycobiome in patients with severe acute pancreatitis and its association with mortality

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Abstract

Background The dysbiosis of gut microbiome is known to have a significant impact on the progression of sever acute pancreatitis (SAP). This study intends to investigate the alteration of gut microbiome in SAP patients during hospitalization and its association with mortality. Bacterial and fungal compositions of the fecal microbiota were determined via 16S and ITS1 sequencing, respectively.

Results Our findings indicated that SAP patients exhibited bacterial and fungal dysbiosis either at the time of admission or within the first 72 h of admission prior to any antibiotic treatment, characterized by reduced biodiversity, lower abundances of *Blautia*, *Bifidobacterium*, and increased *Escherichia-Shigella*, *Enterococcus*, and *Candida*. The overall microbiome was partially restored post-treatment in survivors, including commensal *Bifidobacterium* enhancement. In contrast, non-survivors experienced sustained perturbations during hospitalization, with over-representation of pathogenic *Enterococcus* and *Candida*. A classifier based on 20 optimal bacteria markers showed superior efficiency for predicting mortality. Interestingly, the abundances of *Acinetobacter*, *Klebsiella* and *Candida* were considerably elevated in the gut of patients who subsequently developed infectious complications.

Conclusions Our study provided a comprehensive profile of gut bacteriome and mycobiome in SAP patients and identified the temporal trajectory alterations of microbiome associated with mortality. These findings underscore the importance of early recognition of pathobiome states and the potential role for the modulation of microbiota during the development of SAP.

Keywords Severe acute pancreatitis, Gut microbiome, Mycobiome, Pathobiota, Infection, Short chain fatty acids

[†]Cong He and Jinyun Wang joint first co-authorship.

Common definitions of random forest classifiers, network diameter, modularity, closeness centrality, edge numbers, alpha diversity, beta diversity, 16S rRNA gene sequencing, ITS rRNA gene sequencing, OTU, Sobs, Shannon index, Chao1 index, can be found in the Supplementary materials.

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Introduction

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas associated with substantially morbidity and mortality [1]. While most patients experience a mild disease course, approximately 20% develop severe pancreatitis (SAP), characterized by necrosis of the (peri) pancreatic tissue and/or (multiple-)organ failure [2]. In addition to respiratory, circulatory and kidney failure, gut injury is also an important concern in SAP patients. About 40% of patients experience gut failure, which is independently associated with higher mortality rates [3]. Gut failure often leads to impaired intestinal barrier function, facilitating bacterial and fungi translocation from the gut and subsequent development of infectious complications [4]. Infected pancreatic necrosis, one of the most severe complications of SAP with a mortality rate of up to 35%, is mainly attributed to bacteria originating from the gut [5]. Therefore, the gut not only suffers damage as a victim but also contributes a second hit to the progression of SAP.

Accumulating evidence suggests imbalances in gut bacteria occur either before or early within the first 3 days after establishment of AP, and the causal role of gut bacteria in AP has been further demonstrated through fecal microbiota transplantation from AP donors to germ-free mice [6, 7]. However, little information is available regarding the trajectory of microbiota changes during AP progression and whether these microbial features are associated with clinical outcomes. In addition to bacteria, it is increasingly evident that fungi are essential components of the gut microbial community, and play a critical role in regulating host homeostasis and physiological processes [8]. To date, there is still a lack of comprehensive understanding regarding the composition, community structure, and interplay between gut mycobiome and bacteria in the context of AP.

In this study, we conducted an observational cohort study of SAP patients admitted to pancreatic intensive care unit. Stool samples were collected from patients upon admission to discharge for 16S rRNA and ITS1 sequencing. This allowed us to decipher the temporal changes in gut bacteria and fungi throughout their hospitalization. Additionally, we also examined whether the abundances of specific taxa were associated with the mortality outcome and the occurrence of infectious complications.

Methods

Study design and participants

This was a prospective observational study conducted in the pancreatic intensive care unit of the First Affiliated Hospital of Nanchang University, China from May 2019 to December 2020. Adult patients aged 18–75 who were diagnosed with SAP and admitted to pancreatic ICU within 72 h after the onset of symptoms were enrolled. SAP was defined in accordance with the revised Atlanta classification [9]. Exclusion criteria included (1) treatment with antibiotics or probiotics before the first sample was collected; (2) length of hospital stay less than 7 days; (3) history of abdominal surgery; and concomitant with (4) pregnancy; (5) gastrointestinal disease; (6) malignant cancer. Acute Physiology and Chronic Health Evaluation (APACHE-II) and Sequential Organ Failure Assessment (SOFA) scores were recorded by specialized pancreatic ICU physicians at admission. Basic information was also obtained from each patient, including age, sex, body mass index (BMI), etiology, history of smoking and drinking, antibiotic usage, infectious complications, and mortality within 90 days. The healthy volunteers who met the following inclusion criteria were recruited from the physical examination center: (1) asymptomatic; (2) had not taken antibiotics or probiotics within 4 weeks; (3) not pregnant; and free of chronic (4) metabolic; (5) cardiovascular; (6) gastrointestinal diseases. Age- and sex-matching processes was performed to select comparable controls. Written informed consent was obtained from each participant following protocols approved by the Medical Ethics Committee of the an First Affiliated Hospital of Nanchang University (No. 2019055). This study was retrospectively registered with the Chinese Clinical Trial Registry (registration number PID 283891).

Fecal sample collection and processing

The collection of fecal samples from the patients started within 72 h after their admission and continued weekly until they were discharged or deceased. The first samples collected were labeled as T1, which represented the acute phase of the patients' condition. The final samples collected were labeled as T3, indicating the endpoint of the patients' treatment. The samples collected between T1 and T3 were labeled as T2, representing the progression stage of the patients' condition. Stool samples from healthy individuals were collected once as the control group. Immediately following defecation, fresh stool samples were divided into three parts and frozen at −20 ℃, and then stored at −80 ℃ until analysis.

Bacteria, fungi and short chain fatty acids analysis

Microbial genomic DNA was extracted from fecal samples and amplified using primers of bacterial 16S rRNA gene and the fungi internal transcribed spacer (ITS) rRNA gene. Purified amplicons were pooled in equimolar amounts and then paired-end sequenced (2 × 300) on an illumina Miseq PE300 platform (Illumina, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw sequencing reads were demultiplexed, quality-filtered by Fastp version 0.19.6 and merged by FLASH version 1.2.11 [10, 11].

Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE version 11 and chimeric sequences were removed [12]. The taxonomy of each OTU representative sequences was analyzed by RDP Classifier version 2.13 against Silva 16S rRNA database (v138) for bacteria and UNITE (8.0) for fungi using confidence threshold of 0.7 [13]. The microbial data was analyzed on the Majorbio Cloud Platform (http://www.majorbio.com/) [14]. Alpha diversity indexes, including Sobs, Shannon and Chao1, were calculated by Mothur v.1.30.2 to evaluate the richness and evenness of gut microbiome. Beta diversity was measured by partial-least squares discriminant analysis (PLS-DA) using R (v3.3.1) and permutational multivariate analysis of variance (PERMANOVA) was applied to figure out whether the microbiome composition was significantly different among different groups. The microbial taxonomic distributions of each group were visualized using R (v3.3.1). Wilcoxon signed rank-sum test was used to analyze the differential taxa for paired samples. Significant taxa were reported at FDR-adjusted $p < 0.05$.

Co-occurrence network with nodes representing 97% cutoff OTUs and edges representing correlations between these OTUs was constructed using R package igraph. To eliminate the effects of random sampling, we repeated the operations for 1000 times echo network. We performed principal component analysis taking topological features of number of vertices, number of edges and modularity as input. We chose the representative network, whose score on the first principal component axis was the closest to the mean value of all the networks. The two representative networks were explored and visualized with the interactive platform gephi. The nodes in networks represent OTUs and the edges that connect these nodes represent correlations between OTUs. Network modularity was recalculated using the gephi built-in algorithm with a resolution of 8. The Sankey plot was drawn using R library "ggalluvial".

We used random forest classifier to predict sample type, taking stool community composition of bacteria and/or fungi, or four SCFAs as features. All available timepoints (T1-T3) were included in the random forest analysis to maximize the sample size. The genera composition data of bacteria and fungi were normalized separately before analyses. To filter out redundant features, up to 20 variables were selected by using the minimum Redundancy Maximum Relevance (mRMR) filterranking algorithm, using the R package "mRMRe" [15]. We use nested cross validation to get feature importance scores and sample predictions for all samples using the classify-samples-ncv pipeline in qiime2 (2019.4) [16]. Receiver operating characteristic (ROC) figures were drawn using R package "pROC" [17].

Short chain fatty acids (SCFAs) determination

Fecal samples (25 mg) were complemented with 500 µl of water containing 0.5% phosphoric acid and 50 µg/ml of 2-ethylbutyric acid, and then extracted according to the manufacturer's protocol (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). The contents of SCFAs were determined using an Agilent 8890B-5977B GC–MS system (Agilent Technologies Inc., USA) equipped with a HP FFAP column (30 m × 0.25 mm × 0.25 µm, Agilent J&W Scientific, USA). The ion fragments of target SCFAs were automatically identified by MassHunter quantitative software (Agilent Technologies Inc., USA, No. V10.0.707.0), and the SCFAs concentrations were calculated by standard curve. SCFAs standards were mixtures of acetic acid, propanoic acid, butyric acid.

Statistical analysis

SPSS 26.0 software was used for statistical analysis and $p < 0.05$ was considered statistically significant. Measurement data conforming to normal distribution were expressed as Mean ± SD. If the variance was homogeneous, two independent samples T test were selected for comparison. For variables that were not normally distributed, the approximate T test was carried out and the measurement data of skew distribution was expressed by median (quartile method). Mann–Whitney U test was performed to compare nonparametric variables of two sample groups, while multiple group comparisons were made using Kruskal–Wallis test.

Results

Patient enrollment and characteristics

A total of 73 SAP patients admitted to pancreatic ICU within 72 h and 28 healthy volunteers were enrolled in this study. The flow chart illustrating the process of patient screening was shown in Fig. 1. The most common cause of SAP among the enrolled patients was hypertriglyceridemia (HTG), accounting for 41% of cases. Biliary pancreatitis accounted for 33% of cases, followed by alcoholic pancreatitis at 19%. Out of the 73 patients, 17 (23%) died within 90 days after admission, while 56 (77%) were survivors. Patients' characteristics were summarized in Table 1. Age and sex were matched between the patients and healthy controls ($p > 0.05$). Compared to survivors, non-survivors were older and had higher APACHE-II score and infection rates (Table S1).

Early dysbiosis of bacterial and fungi at the onset of SAP

A total of 12,315,418 reads were obtained from 16S rRNA gene sequencing and ITS1 sequencing yielded 17,311,489 reads. To elucidate the initial alterations of gut microbiome, we first analyzed the stool samples from SAP patients at baseline (T1). Analysis of alpha diversity revealed that both the richness and diversity, as

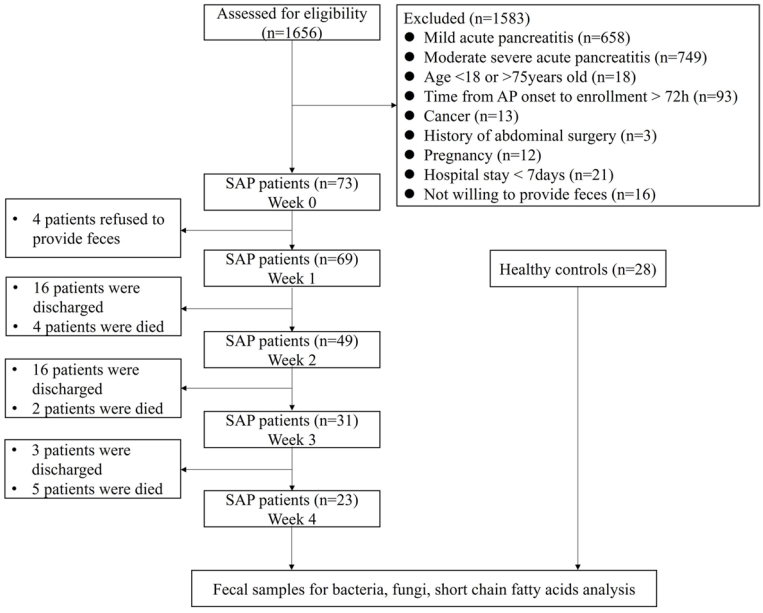

Fig. 1 Flow diagram of the patient selection process and sample collection analysis. SAP, severe acute pancreatitis

Table 1 Basic demographics of patients and healthy subjects

	SAP (*n* = 73)	Healthy controls (*n* = 28)	*p* value
Age, median years (IQR)	47(37–59)	49(43–56)	0.56
Sex, male/female	49/24	13/15	0.253
BMI, median kg/m^2 (IQR)	25(24,28)	23(20,25)	0.652
Etiology, n (%)			
HTG	30(41%)		
Biliary	24(33%)		
Alcohol	14(19%)		
Other	5(7%)		
APACHE-II score, median (IQR)	11(9–15)		
SOFA score, median (IQR)	4(3–6)		
Infectious complications (%)	45(62%)		
Hospital stay, days, median (IQR)	19(13–39)		
ICU stay, days, median (IQR)	12(7–25)		
Mortality (%)	17(23%)		

HTG Hypertriglyceridemia, *APACHE II* The Acute Physiology and Chronic Health Evaluation II score, *SOFA* The Sequential Organ Failure Assessment score

calculated by Sobs, Shannon and Chao1 indices, of bacteria and fungi were remarkably lower in SAP patients than in healthy controls (HC) (Fig. 2A-B). Partial least square discriminant analysis (PLS-DA) plot showed that samples of SAP and healthy subjects were separated into two strikingly distinct groups in both bacteria and fungi community (PERMANOVA, $R^2 = 0.075$, $p = 0.001$ for bacteria; $R^2 = 0.070$, $p = 0.001$ for fungi) (Fig. 2C-D).

In keeping with previous work, we found an increase in the bacterial phylum Proteobacteria and a decrease in Firmicutes and Actinobacteriota in SAP patients compared to the control group (Fig. 2E). Furthermore, SAP group was dominated by pathogenic genus *Escherichia-Shigella*, *Enterococcus*, and *Klebsiella*, the abundances of which were up to 49%, compared to only 11% in healthy subjects (Fig. 2G-H). Conversely, the potential beneficial genera such as *Blautia*, *Bifidobacterium*, and *Romboutsia,* were statistically less abundant in SAP patients than in healthy controls (Fig. 2G).

In the analysis of the fungal microbiota, we found that the gut mycobiome was primarily comprised of two phyla: Ascomycota and Basidiomycota. A notable feature was the increased abundance of Ascomycota in SAP patients, which was balanced by an equivalent decrease in Basidiomycota (Fig. 2F). At the genus level, *Candida*, *Penicillium*, *Saccharomyces*, and *Aspergillus* were the most abundant fungi (Fig. 2J). Upon specific analysis, we noted that SAP patients had higher proportions of *Candida* compared to healthy controls (Fig. 2I). On the contrary, *Saccharomyces* and *Aspergillus* were more abundant in healthy individuals than in SAP patients (Fig. 2I). Altogether, early dysbiosis of gut bacterial and fungal communities was observed at the onset of SAP.

Dynamic changes in the gut bacteria and its metabolite short chain fatty acids during hospitalization

Next, we classified the patients into two groups: survivors (S) and deceased (D), and then examined the changes of gut bacterial composition from early after admission at T1, to progression (T2 and T3). In brief, both richness and diversity values were lower at T2 and T3 compared to T1 (Fig. 3A-B). The Sobs index, a measure of species abundance, declined more sharply in non-survivors compared to the survivors (Fig. 3A). Similarly, the Shannon

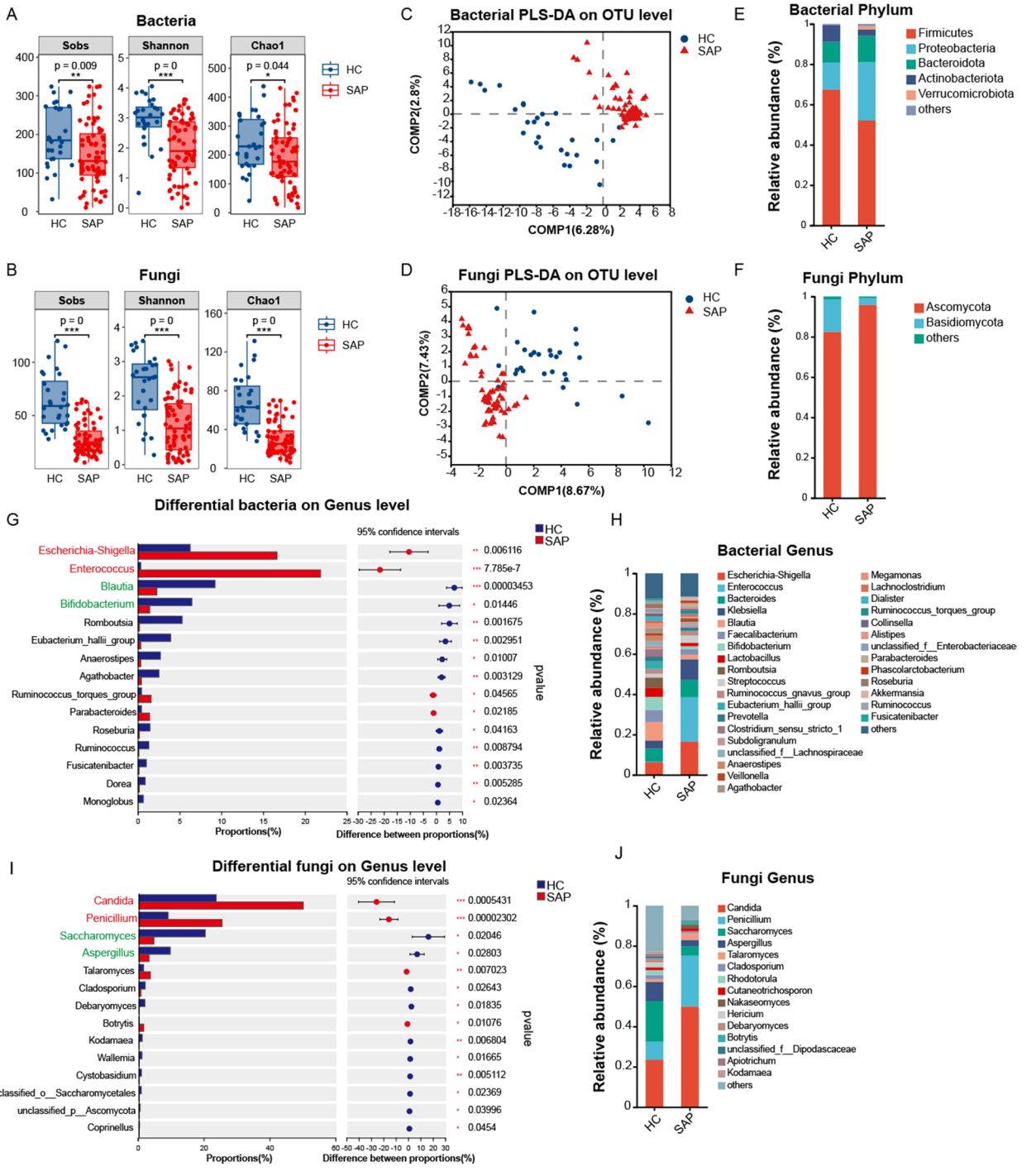

Fig. 2 Gut microbial variations in individuals with SAP. Alpha diversity of bacteria (**A**) and fungi (**B**) represented by observed species (Sobs), Shannon, Chao1 indices in SAP patients and healthy control (HC) individuals (Wilcoxon rank-sum test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Partial least squares discriminant analysis (PLS-DA) of bacteria (**C**) and fungi (**D**) revealed compositional variations between SAP and HC. Relative abundance of bacterial and fungi at phylum (**E**, **F**) and genus (**H**, **J**) level. Bacterial (**G**) and fungi (**I**) genera with significant differences (Wilcoxon rank-sum test with Benjamine-Hochberg correction; FDR [false discovery rate] < 0.05) between SAP and HC. SAP, severe acute pancreatitis

index, which measures species diversity, continuously dropped in the non-survivors, while it remained relatively stable in the survivors (Fig. 3B). When using PLS-DA and PERMANOVA on the basis of Bray–Curtis distance, separation among samples from T1, T2 and T3 was observed in survivor patients ($R^2 = 0.04$, $p = 0.001$) (Fig. 3C). However, no significant differences were noted in the T1-T2-T3 distances within the D group, which

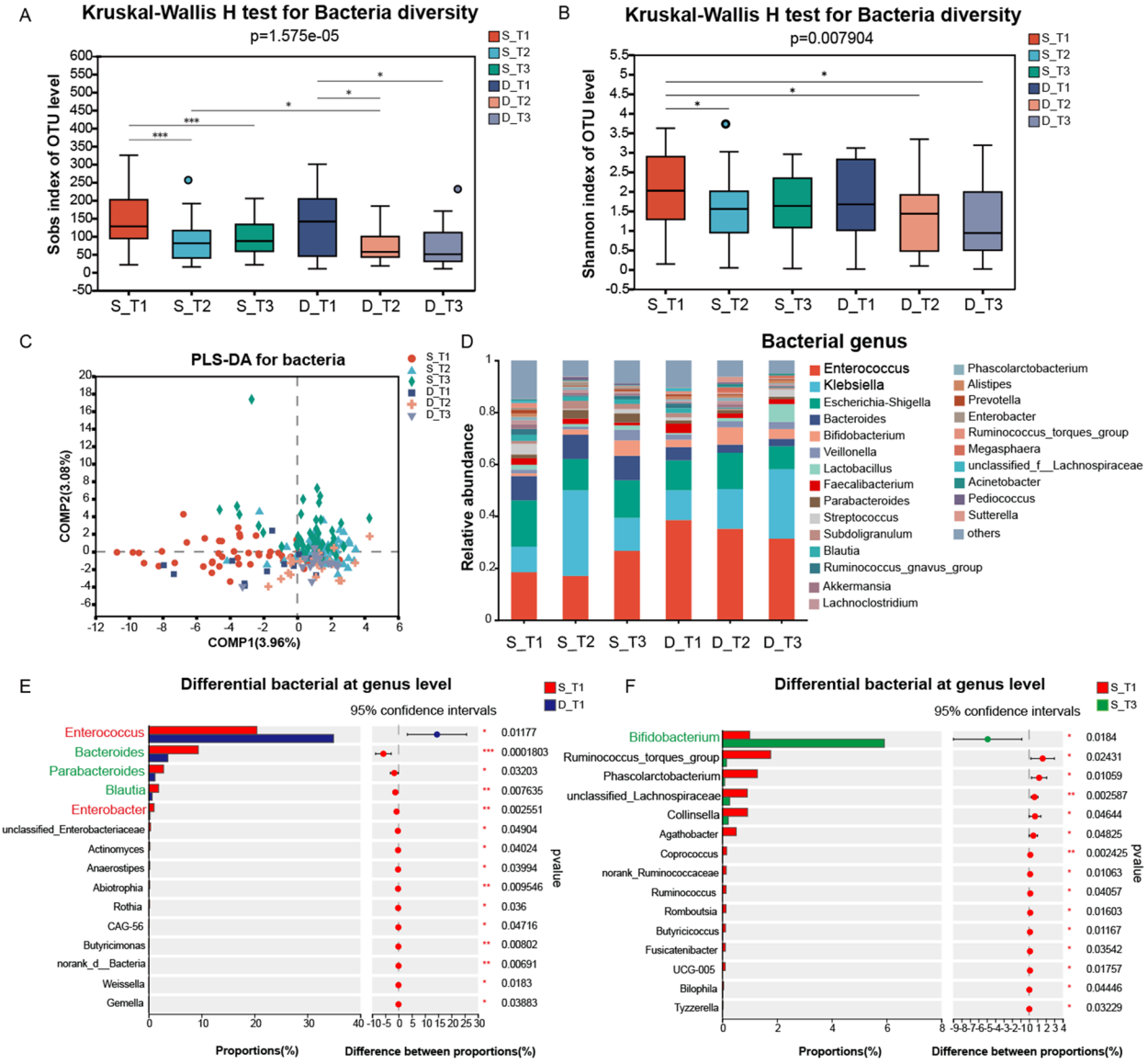

Fig. 3 Gut bacteriome alterations in SAP patients with different clinical outcomes during hospitalization. Stool samples were collected from admission (T1) through progression (T2) to discharge (T3). Patients were divided into survivors and non-survivors according to their prognosis during 90-day follow-up. **A**-**B** Comparison of alpha diversity among different groups. **C** Profile of bacterial taxonomy was shown by PLS-DA. **D** Relative abundance of bacterial genera between survivors and non-survivors at different time points. **E** Bacterial genera with significant differences between survivors and non-survivors at admission (Wilcoxon rank-sum test with Benjamine-Hochberg correction; FDR < 0.05). **F** Differential bacterial genera between admission (T1) and discharge (T3) in survivors. S, survivors; D, non-survivors; T1, admission stage; T2, progression stage; T3, discharged stage. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

indicates a comparatively lesser microbial variation in non-survivor patients.

To explore differences in microbial variations across different disease stages, we compared the gut microbiota composition between survivors and non-survivors at the time of admission and during progression. In survivors, a notable depletion in the Firmicutes/Proteobacteria ratio was observed at T1 (admission), with a subsequent recovery by T3 (recovery). In contrast, no significant change in this ratio was found in the non-survivors (Fig. S1). At the genus level, *Enterococcus* was statistically more abundant in the non-survivors compared to the survivors, while *Bacteroides* was less abundant (Fig. 3E). Among survivors, the relative abundance of *Klebsiella* was higher in the T2 (progression stage) samples than in the T1 (admission stage) samples. It was then found to be lower once again in the T3 (recovery stage) samples (Fig. 3D). In contrast, the D group patients had higher levels of *Klebsiella* in the end stage samples compared to the admission and progression stage samples (Fig. 3D). Moreover, the abundance of *Bifidobacterium* was remarkably elevated in the T3 (recovery stage) samples from survivor

patients compared to those collected at T1 and T2 stages (Fig. 3F). Of note, the overgrowth of *Enterococcus*, which was observed in patients compared to healthy subjects, was much more profound in non-survivors when compared to survivors at the time of admission (Fig. 3E).

Short chain fatty acids (SCFAs) are widely recognized as beneficial metabolites produced by the gut microbiota. However, their changes in patients during hospitalization have not been fully understood. To determine the alterations of SCFAs from admission to discharge, we performed a paired comparison between survivors and non-survivors. The levels of SCFAs, especially acetic acid and propionic acid, were found to increase in recovering patients (Fig. S2). In contrast, no significant variation was observed in the non-survivors. Collectively, the restoration of SCFAs is associated with the good prognosis in SAP patients.

Temporal changes in the gut fungi over time of hospitalization and its association with clinical outcome

The alpha diversity indexes, including Sobs and Shannon, remained similar throughout the hospital stay for both survivors and non-survivors (Fig. 4A-B). However, beta diversity determination using PLS-DA and PERMANOVA revealed significant differences in fungal composition with the recovery of SAP in survivors ($R^2 = 0.02$, $p = 0.003$) (Fig. 4C), suggesting that the fungal community changed during that recovery phase in these patients. In contrast, no such response was noted in non-survivor patients, indicating a persistent dysbiosis. Non-survivors displayed a higher abundance of *Candida*, which reached up to 60%, throughout the entire course of the disease compared to survivors (Fig. 4D). Specifically, the pathogenic genera *Penicillium*, *Talaromyces*, and *Botrytis*, which were overrepresented in SAP patients compared to healthy subjects, were statistically reduced at the last follow-up in survivors. There were no such alterations in non-survivors (Fig. 4H-J). In comparison to survivors, the non-survivors exhibited persistent fungi dysbiosis from admission onwards, characterized by an overgrowth of pathogenic *Candida*, especially *Candida albicans*, and a loss of commensal *Cladosporium* (Fig. 4E-G). These findings imply that the divergent alterations of gut fungi across the course of SAP are correlated with disease prognosis.

Bacteria-fungi co-occurrence network differ in survivors and non-survivors

To elucidate whether the microbiome co-occurrence pattern is associated with disease progression, we analyzed the interkingdom between bacteria and fungi in patients with favorable and poor prognosis. In the S (survivor) group, module 6, 1, and 11 were primarily represented, with relatively equal proportions. In contrast, the D (non-survivor) group was dominated by module 4 (Fig. 5A). The modularity index, which measures how well a microbiome network is organized into separate functional teams, dramatically decreased from 0.639 in survivors to 0.379 in non-survivors across all time points during hospitalization (Fig. 5D). Various network indices, including the number of nodes, edges, average degree, closeness centrality, degree centrality, and density were higher in the non-survivors than the survivors. This suggests stronger interactions within the microbiome during disease deterioration (Fig. 5B, D, Fig. S3). The average path distance and diameter were statistically reduced in the D group, indicating a high degree of interconnectedness and efficient communication within this network (Fig. S6). A higher betweenness centrality value in the D group also suggests frequent flow of information and resources among nodes in the network (Fig. S3). Interestingly, fungi made up 6% of the nodes in the survivors, but this proportion rose dramatically to 14% in the non-survivors (Fig. 5C). The module 16 in the survivors was totally composed of fungi, which positively correlated with each other and relatively independent from other modules. In contrast, the fungi in the non-survivors were distributed in four modules, including module 4, 12, 1, and 6 (Fig. 5C). Taken together, patients with favorable prognosis exhibited distinct bacteria-fungi co-occurrence patterns compared to those with poor outcomes.

Identification of gut bacteria, fungi and short chain fatty acids associated with mortality risk

To identify the characteristics of the gut microbiome associated with mortality after ICU admission, random forest models were constructed at the genus level for both bacteria and fungi, as well as for SCFAs. We assessed the efficacy of these classifiers in distinguishing between survivors and non-survivors. At the genus level, random forest classifiers based on the top 20 most important bacteria and fungi achieved area under the receiver operating characteristic curve (AUC) values of 0.812 and 0.657, respectively (Fig. 6C-D). This suggests that the bacterial classifier performed better in differentiating prognosis than the fungi one. For the bacterial classifier, *Enterococcus*, which was predominant in patients, was a highly labeled microbiota followed by *Bacteroides* between the S (survivor) and D (non-survivor) groups (Fig. 6A). For the fungal classifier, the major features included *Candida*, *Penicillium*, *Saccharomyces* and *Aspergillus*, although their predictive efficiency was relatively low (Fig. 6B). However, the model based on SCFAs metabolites achieved the lowest AUC value of 0.503 for determining mortality compared to the bacterial and fungal models (Fig. 6E).

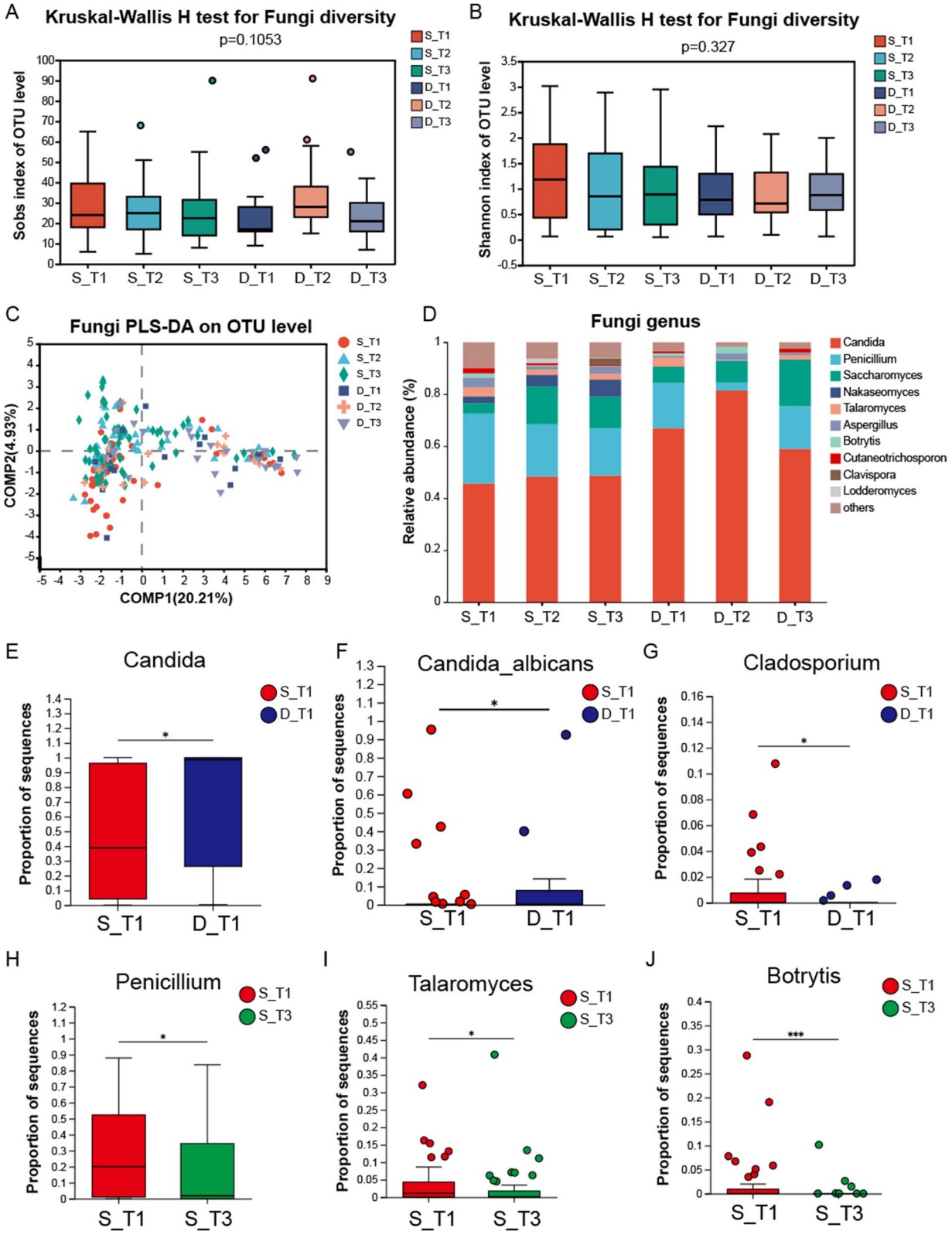

Fig. 4 (See legend on next page.)

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Fig. 4 Temporal changes of gut mycobiome in SAP patients with different outcomes during hospitalization. Alpha diversity indexes, including Sobs (**A**) and Shannon (**B**), were calculated in survivor patients and non-survivor patients from T1 (admission) through T2 (progression) to T3 (discharge). **C** Beta diversity of mycobiome variation between S and D group across different stages. **D** Relative abundance of fungal genera. Differential proportion of sequences attributable to *Candida* (**E**), *Candida albicans* (**F**), *Cladosporium* (**G**) between survivors and non-survivors at admission. Discrepant proportion of sequences attributable to *Penicillium* (**H**), *Talaromyces* (**I**), *Botrytis* (**J**) between admission and discharge in survivors. S, survivors; D, non-survivors; T1, admission stage; T2, progression stage; T3, discharged stage. *$p < 0.05$, ***$p < 0.001$

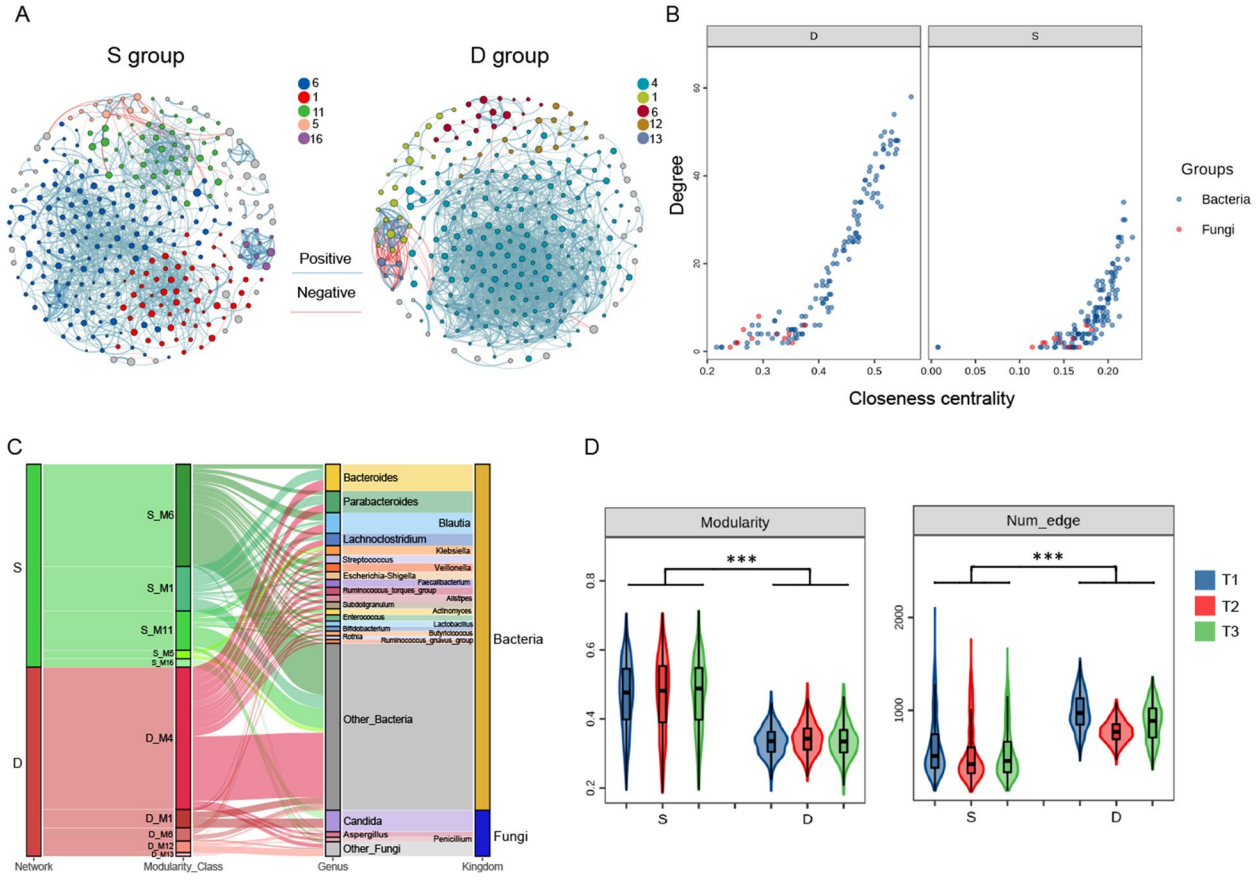

Fig. 5 Interkingdom co-occurrence networks. **A** Networks contained both bacterial and fungal taxa showing a more balanced pattern of modular distribution in the survivors compared to those in the non-survivors. Modules represented by nodes with different colors. Blue and red colors of the edges indicated positive and negative correlations, respectively. **B** Comparison of node-level topological features (degree and closeness centrality) demonstrating the higher degree and closeness centrality values for the hub taxa in non-survivor group than survivor group. **C** Sankey diagram of bacteria and fungi composition attributed to different modules of survivor and non-survivor group at the genus level. **D** Lower modularity and higher edge numbers were observed in non-survivors than those in survivors during hospitalization. T1, admission stage; T2, progression stage; T3, discharge stage; S, survivors; D, non-survivors. ***$p < 0.001$

Associations between gut microbiota and infectious complications

Infectious complications are considered to be the most severe complications of SAP often leading to late mortality. The gut is thought to be the primary source of these infections [18]. The definitions of infectious complications were established according to a previous study [19]. We evaluated infection based on the occurrence of at least one infectious episode in 45 patients (62%) (Table S2). Pancreatic necrosis was the most common type of infection, with an incidence rate of 48% (35/73). This was followed by bacteremia at 33% (24/73), pneumonia at 21% (15/73), infected ascites at 14% (10/73), and urinary tract infections at 1% (1/73). Patients who developed infections had higher mortality rates than those without infections (38% vs 0%, $p = 0.000$). We identified 78 strains of microorganisms from 45 SAP patients with infectious complications. Gram-negative bacteria accounted for 50% of these strains, primarily including *Acinetobacter baumannii*, *Klebsiella pneumonia* and *Enterobacter spp.*. The main Gram-positive bacteria were *Enterococcus spp.* and *Staphylococcus spp.*. Thirteen patients were found to

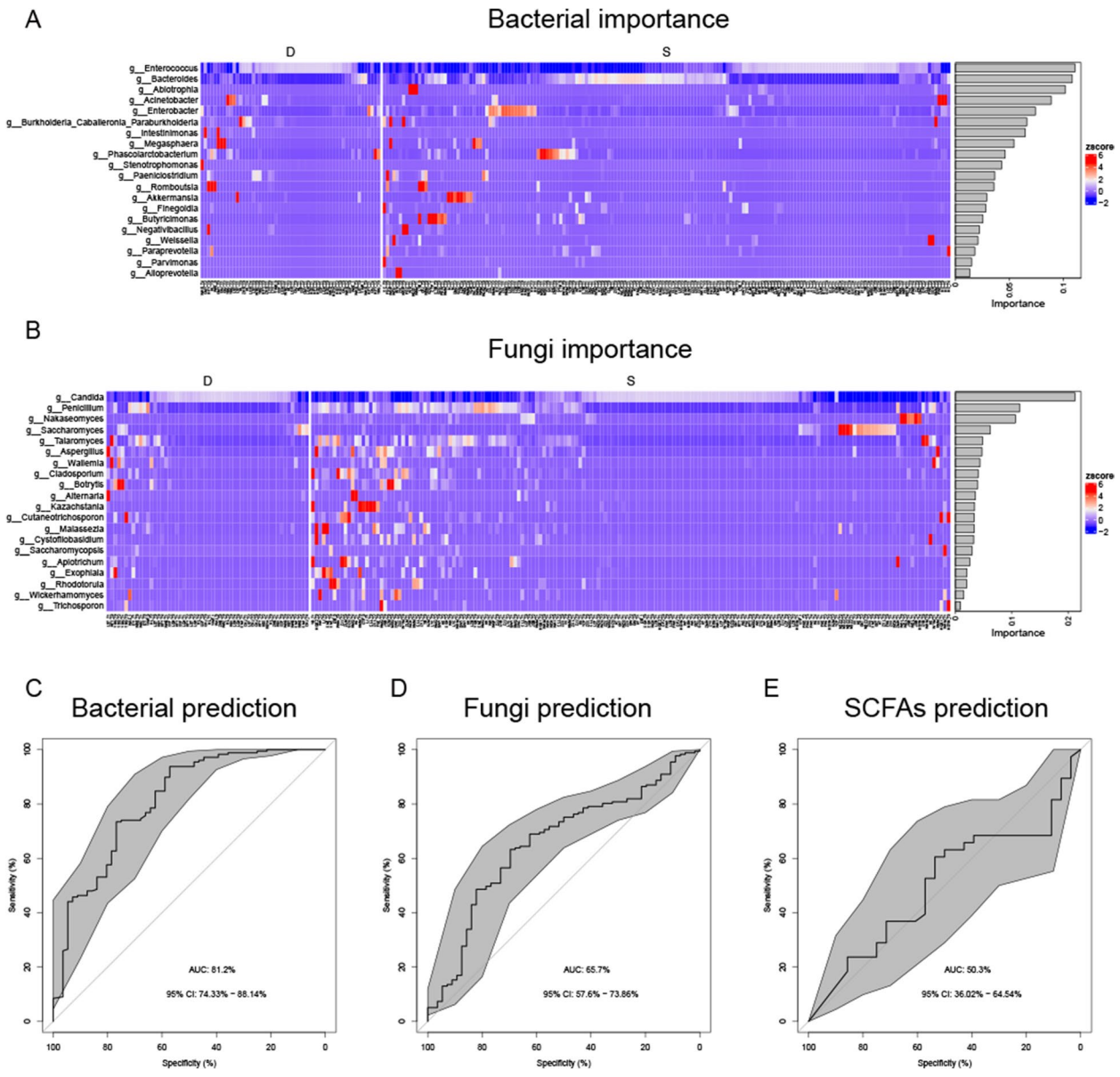

Fig. 6 Identification of gut bacteria, fungi and short chain fatty acids classifier for mortality in SAP. The top 20 main important mortality associated bacteria (**A**) and fungi (**B**) at genus level from random forest models. **C**-**E** Receiving operational curve (ROC) analysis showed bacterial markers achieved better accuracy for predicting mortality, achieving an area under curve (AUC) value of 0.812, compared to fungi (AUC: 0.657) and short chain fatty acids (SCFAs) (AUC: 0.503). S, survivors; D, non-survivors

have fungal infections, primarily *Candida*. Among the 45 patients with infectious complications, 13% (6/45) had infections with multi-drug resistant (MDR) bacteria.

To determine if gut microbiome dysbiosis is associated with infection complications, we compared the admission samples (T1) between patients with and without infection. While the Sobs and Chao1 indices of bacteria reduced substantially in infected patients, the alpha diversity indexes of fungi did not show significant differences between the two groups (Fig. 7A, Fig. S4A). Permutational analysis of variance (PERMANOVA) showed significant compositional differences in both bacterial ($R^2 = 0.016$, $p = 0.003$) and fungal ($R^2 = 0.011$, $p = 0.01$) communities between the infected and non-infected groups (Fig. 7B, Fig. S4B). Notably, the relative abundances of opportunistic pathogens, such as *Acinetobacter*, *Klebsiella*, *Candida tropicalis*, were greatly expanded in the gut of the infected group compared to the non-infected group. In contrast, the beneficial probiotic *Bifidobacterium* was depleted in the infected group (Fig. 7E-H).

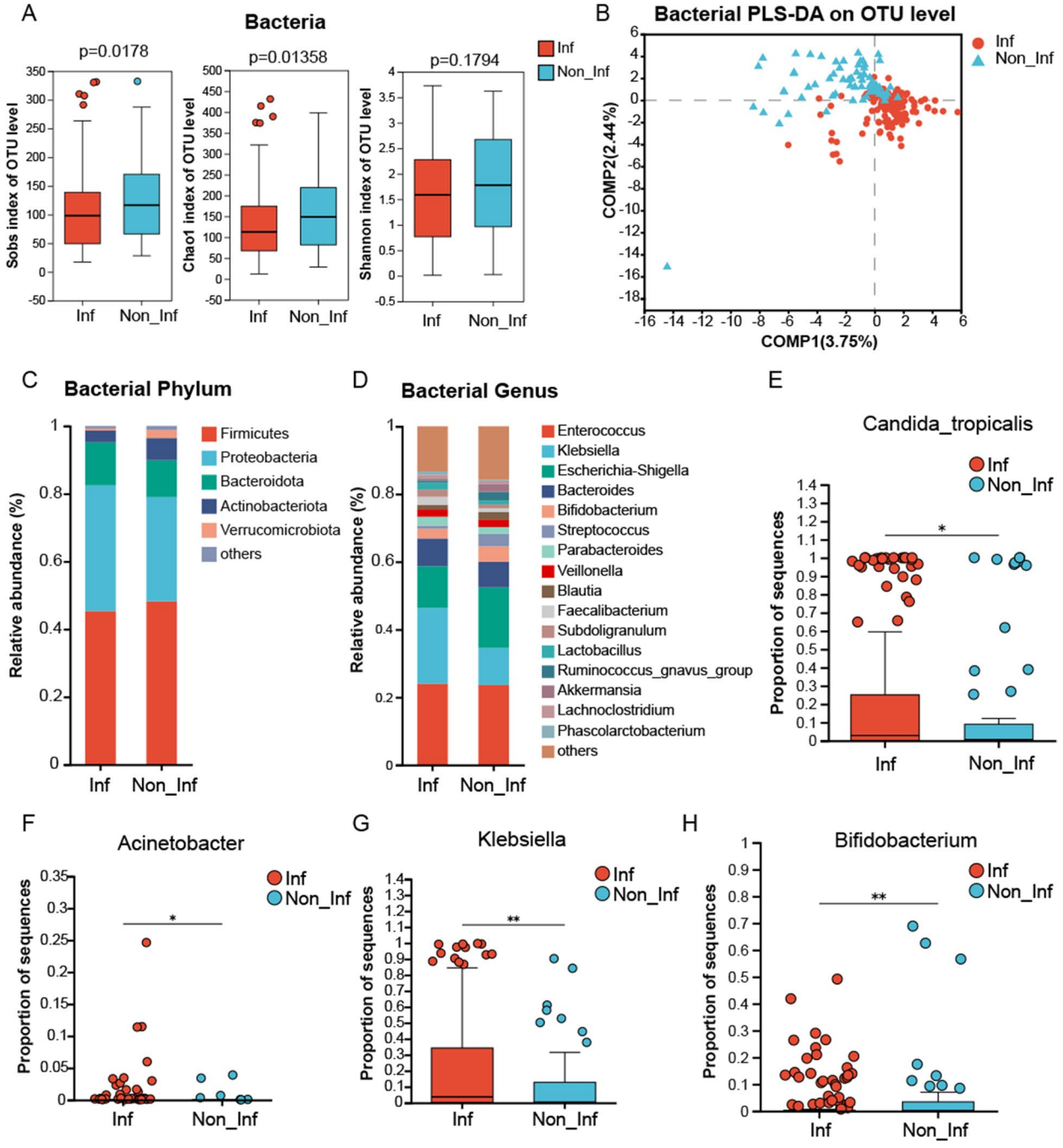

Fig. 7 Dysbiosis of the gut bacteria and fungi in SAP patients with infectious complications. **A** Bacterial alpha diversity, as revealed by observed species, Shannon, and Chao1, comparing infected and non-infected patients during hospitalization. **B** PLS-DA plot showing compositional differences of bacteria in beta diversity between infected and non-infected patients. Bacterial composition at the phylum (**C**) and genus (**D**) level. **E** Fungi species *Candida tropicalis* was significantly enriched in the gut of infected patients compared to those without infections. Differential bacterial genera, including *Acinetobacter* (**F**), *Klebsiella* (**G**), *Bifidobacterium* (**H**), between infected and non-infected patients. Inf, infected patients; Non_Inf, non-infected patients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Discussion

The present study is among the pioneering attempts to explore the characteristics of gut bacteria and fungi in patients with SAP at various disease progression stages and with different clinical outcome (survivors and non-survivors). We found that SAP patients experienced gut dysbiosis, characterized by reduced biodiversity and disordered community structure, affecting both bacterial and fungal communities when compared to healthy individuals. Furthermore, we observed dynamic changes in

bacterial and fungal populations in survivors across three stages (admission, progression, and discharge), but the non-survivors exhibited persistent microbial disturbance without response to treatment. Random forest analysis showed that the bacterial signature demonstrated superior performance in predicting mortality compared with fungi and SCFAs signatures. Additionally, we noted an overgrowth of certain pathogens, including *Klebsiella*, *Acinetobacter*, and *Candida*, in the gut of patients with infectious complications, suggesting that microbiome dysbiosis might be the origin of infection.

Emerging evidence suggests that gut bacteria change dramatically either before or at the onset of AP, however, their alterations throughout hospitalization remain unclear [7]. Our study revealed a decrease in bacterial diversity and an increase in proportions of pathogenic *Escherichia_Shigella* and *Enterococcus* when compared to healthy individuals, consistent with previous reports [6, 20, 21]. *Escherichia coli*, an *Escherichia* commensal organism, has been reported to aggravate acute necrotizing pancreatitis by damaging the intestinal barrier [22]. In our study, we noted increased bacterial disturbance in patients with poor outcomes apparently within 72 h after the onset of SAP, including a flourish of *Enterococcus* and a loss of *Parabacteroides*. In another study by Yu et al., an increased abundance of *Enterococcus* was observed in patients with SAP compared to patients with mild AP [20]. *Enterococcus faecium* has been shown to be one of the best discriminators between necrotizing patients and non-necrotizing ones, and it might be a potential predictor for infection [23]. Moreover, an elevation of *Parabacteroides* might protect against the exacerbation of SAP by producing acetate [24]. We also noted that while bacterial diversity tended to restore at discharge in the survivor group, it progressively decreased in non-survivors. A notable loss of microbial diversity has been documented in critically ill patients and is independently associated with death during ICU stay [25]. Moreover, we observed a restoration of *Bifidobacterium* in survivors at discharge compared to the admission stage. A lower abundance of *Bifidobacterium* has been reported to be associated with AP severity, and its metabolite can inhibit pancreatic and systemic inflammation [26]. Levels of SCFAs, which have been proven to stabilize the intestinal barrier and improve pancreatic injury, were found to increase in survivors, likely due to the rejuvenation of SCFAs-producing bacteria [27]. While both our findings and existing evidence implicate gut dysbiosis in AP progression, the randomized double-blind PROPATRIA trial demonstrated that probiotic prophylaxis not only failed to reduce infection rates but unexpectedly increased mortality [19]. This unexpected outcome may be attributed to probiotic-induced gut ischemia and local inflammation in critically ill patients. Thus, future studies are warranted to investigate strain-specific effects, optimal dosing, and timing of administration, with a prioritized focus on safety-especially in patients with systemic inflammation or organ failure.

Dysbiosis of the human gut mycobiome has been associated with a variety of diseases, but its role in SAP remains obscure. This study, to our knowledge, is the first to characterize the gut mycobiome in SAP and to elucidate mycobiota alterations during hospital stay in relation to prognosis. Patients with SAP displayed enteric fungal dysbiosis, characterized by a decrease in biodiversity compared to healthy individuals. The phyla Ascomycota and Basidiomycota were predominant in both SAP patients and healthy subjects, but the ratio of Ascomycota to Basidiomycota was higher in SAP patients. We identified an overgrowth of opportunistic pathogens in SAP patients, such as *Candida tropicalis*. *C. tropicalis* is increasingly recognized for its epidemiological importance and virulence. It can produce strong biofilms and adhere robustly to epithelial and endothelial cells [28]. Infection with *C. tropicalis* can increase intestinal permeability and exacerbate colitis, likely through the induction of robust Th1/Th17 responses [29]. Conversely, some strains of *Saccharomyces*, including *S. cerevisiae* and *S. boulardii*, are probiotic yeasts that are widely used to treat gastrointestinal disorders such as inflammatory bowel disease and various types of diarrhea [30]. *S. boulardii* has been shown to exert protective effects on acute necrotizing pancreatitis by suppressing inflammatory responses and bacterial translocation [31]. Moreover, we found a significant overgrowth of the pathogen *Candida*, particularly *C. albicans*, in non-surviving patients compared with survivors, indicating its role in mortality. Various studies have reported that fungal pathogens contribute to disturbances of gastrointestinal barrier and trigger inflammation by inducing intestinal epithelial cells to secrete chemotactic factors and pro-inflammatory cytokines [32].

In this study, patients were admitted to pancreatic ICU within 72 h of the onset of SAP, and we found a decrease in diversity along with an enrichment of opportunistic pathogens (*Enterobacteriaceae*, *Enterococcaceae*) over symbiotics (*Lachnospiraceae*, *Ruminococcaceae*, *Bifidobacteriaceae*). These findings are consistent with previous studies on critically ill patients, where similar bacterial imbalances, known as pathobiota, were observed regardless of the primary diagnosis [33, 34]. Commensal bacteria such as *Bifidobacterium* and *Lachnospiraceae* have been shown to play a crucial role in resisting colonization by *Enterobacteriaceae* in the gut [35]. We also observed persistent disruption in the gut mycobiome of ICU patients, characterized by decreased diversity and

a marked increase in *Candida*, which aligns with recent findings [36]. Our study identified *Enterococcus* and *Candida* as the most important bacterial and fungal features, respectively, for predicting mortality. This corroborates previous research suggesting that a predominant colonization of *Enterococcus* and *Enterobacteriaceae* in the intestine upon admission is linked with an increased risk of death or secondary infection [25, 37]. Patients with SAP often develop infections in the later stages of the disease, which potentially contribute to poor prognosis. Numerous studies indicate that the pathogens, including both bacteria and fungi, causing infectious pancreatic necrosis primarily originate from the gut [4]. We noted that the bacteria cultured from infected patients included *Klebsiella pneumoniae* and *Acinetobacter baumannii*, while fungi mainly consisted of *Candida*. We also found these pathogens' relative abundance in the gut of infected patients to be statistically higher than in non-infected ones, suggesting that gut-derived pathogens could be the primary source of infection.

Dietary intake/choice of food is an important factor that determines microbial composition in the human gut. The human mycobiota diversity is influenced by the diet of an individual, including fermented food products, bread, as well as alcoholic beverages [38]. Studies on the decreased intake of bread and beer have been shown to minimize the amount of *Saccharomyces cerevisiae* in human stools [39]. In a controlled study, it was examined that *Penicillium* was correlated with a plant-based diet and *Candida* was enriched in animal-based diet participants [38]. Additionally, studies have shown foodborne fungi, such as *Penicillium* and *Aspergillus*, in more than 60% of stool samples from vegetarians, but these fungal taxa were infrequently found in individuals who consumed a Western diet [40]. Our findings align with a recent study demonstrating that AP patients exhibited reduced fungal diversity and *Penicillium* abundance, accompanied by elevated *Candida* abundance during acute phases [41]. The observed depletion of *Penicillium* and other food-associated genera may reflect disease-related dysbiosis and transient reductions due to fasting/enteral nutrition, as these taxa are commonly diet-derived. However, the consistent enrichment of *Candida* across studies, including ours, supports its role in AP progression. We will integrate dietary metadata and viability-based methods to refine our understanding of gut fungi in future work.

The gut microbiome is a complex ecological system, and the interactions between bacteria, fungi, and their relationships are crucial for maintaining ecosystem stability [42, 43]. Co-occurrence network analysis has become a valuable tool for understanding these ecological interactions and providing insights into community stability based on topological properties. We observed decreased modularity and increased closeness centrality and edge numbers in deceased patients compared to those who were discharged with recovery. In a healthy state, the gut microbiome exhibits a certain level of modularity, where microbial species form cohesive subgroups that interact more strongly within themselves than with other groups. However, it has been suggested that the modularity of the gut microbiome may decrease in disease states [44, 45]. Various factors, such as systemic inflammation and altered immune responses, can lead to the loss of microbial species responsible for maintaining modularity, resulting in a more disorganized and less resilient microbial community [46].

Indeed, it is important to acknowledge the limitations of this study. First, as an observational study with a limited sample size from a single center, the generalizability of our findings may be constrained. The gut microbiota biomarkers identified in this study were not externally validated, nor were they confirmed using alternative methods such as qPCR. Further validation in larger, multicenter cohorts is warranted. Second, while our use of 16S rRNA and ITS1 gene sequencing provided valuable insights into microbial composition, this approach only assesses relative microbial abundance rather than absolute quantification and the taxonomic resolution is lower compared to shotgun metagenomics. Finally, although our prospective, longitudinal design revealed significant associations between gut dysbiosis and clinical outcomes, the underlying mechanisms remain unclear. Future studies incorporating in vivo and in vitro experiments to elucidate the causal relationships and functional consequences of these microbial changes are warranted.

Conclusions

In summary, our study provided insights into the early disturbances in gut bacterial and fungal composition in critically ill patients with acute pancreatitis. Notably, patients with poor prognosis exhibited significant microbial dysbiosis upon admission, particularly characterized by the proliferation of *Enterococcus* and *Candida*, which persisted despite treatment interventions. The presence of opportunistic pathogens such as *Acinetobacter*, *Klebsiella*, and *Candida* in the intestines of patients who subsequently developed infections suggested their origin from the gut. Additionally, gut bacteria outperformed fungi and short-chain fatty acids (SCFAs) in identifying patients at high risk of death. These findings emphasize the need for further research to develop precise strategies for modulating both bacterial and fungal imbalances to reduce the occurrence of infectious complications and improve survival rates in critically ill individuals.

Abbreviations

SAP	Severe acute pancreatitis
AP	Acute pancreatitis
ICU	Intensive care unit
APACHE-II	Acute Physiology and Chronic Health Evaluation
SOFA	Sequential Organ Failure Assessment
BMI	Body mass index
ITS	Internal transcribed spacer
OTUs	Operational taxonomic units
PLS-DA	Partial-least squares discriminant analysis
PERMANOVA	Permutational multivariate analysis
SCFAs	Short chain fatty acids
mRMR	Minimum redundancy maximum relevance
ROC	Receiver operating characteristic
HTG	Hypertriglyceridemia
HC	Healthy control
AUC	Area under the receiver operating characteristic curve
MDR	Multi-drug resistant
qPCR	Quantitative polymerase chain reaction

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

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Authors' contributions

CH, NL, and YZ contributed to the conceptualization of the study design and methodology. YZ contributed to funding acquisition. JW, LD, and XL performed the formal data analyses, which was interpreted by CH. WH, LX, YH, LP, XH, LY and HX contributed to patients' enrollment and sample collection. CH wrote the original draft of the manuscript and all authors contributed critical inputs, edits and revisions to produce the final manuscript.

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Data availability

Raw 16S rRNA and ITS1 sequencing data for all samples have been deposited in the NCBI short read archive under accession number PRJNA800481.

Declarations

Ethics approval and consent to participate

This study was approved by Medical Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 2019055). All subjects gave informed consent to participate in the study.

Consent for publication

Not required.

Competing interests

The authors declare no competing interests.

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References

1. Iannuzzi JP, King JA, Leong JH, Quan J, Windsor JW, Tanyingoh D, et al. Global incidence of acute pancreatitis is increasing over time: a systematic review and meta-analysis. Gastroenterology. 2022;162(1):122–34.
2. van Dijk SM, Hallensleben NDL, van Santvoort HC, Fockens P, van Goor H, Bruno MJ, et al. Acute pancreatitis: recent advances through randomised trials. Gut. 2017;66(11):2024–32.
3. Ding L, Chen HY, Wang JY, Xiong HF, He WH, Xia L, et al. Severity of acute gastrointestinal injury grade is a good predictor of mortality in critically ill patients with acute pancreatitis. World J Gastroenterol. 2020;26(5):514–23.
4. Grewe M, Tsiotos GG, Luque de-Leon E, Sarr MG. Fungal infection in acute necrotizing pancreatitis. J Am Coll Surg. 1999;188(4):408–14.
5. Glaubitz J, Wilden A, Frost F, Ameling S, Homuth G, Mazloum H, et al. Activated regulatory T-cells promote duodenal bacterial translocation into necrotic areas in severe acute pancreatitis. Gut. 2023;72(7):1355–69.
6. Zhu Y, He C, Li X, Cai Y, Hu J, Liao Y, et al. Gut microbiota dysbiosis worsens the severity of acute pancreatitis in patients and mice. J Gastroenterol. 2019;54(4):347–58.
7. Li XY, He C, Zhu Y, Lu NH. Role of gut microbiota on intestinal barrier function in acute pancreatitis. World J Gastroenterol. 2020;26(18):2187–93.
8. Zhang F, Aschenbrenner D, Yoo JY, Zuo T. The gut mycobiome in health, disease, and clinical applications in association with the gut bacterial microbiome assembly. Lancet Microbe. 2022;3(12):e969–83.
9. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102–11.
10. Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884-90.
11. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63.
12. Edgar RC. Uparse: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8.
13. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–7.
14. Ren Y, Yu G, Shi C, Liu L, Guo Q, Han C, et al. Majorbio cloud: a one-stop, comprehensive bioinformatic platform for multiomics analyses. Imeta. 2022;1(2):e12.
15. De Jay N, Papillon-Cavanagh S, Olsen C, El-Hachem N, Bontempi G, Haibe-Kains B. mRMRe: an R package for parallelized mRMR ensemble feature selection. Bioinformatics. 2013;29(18):2365–8.
16. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
17. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.
18. Imam Z, Simons-Linares CR, Chahal P. Infectious causes of acute pancreatitis: a systematic review. Pancreatology. 2020;20(7):1312–22.
19. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet. 2008;371(9613):651–9.
20. Yu S, Xiong Y, Fu Y, Chen G, Zhu H, Mo X, et al. Shotgun metagenomics reveals significant gut microbiome features in different grades of acute pancreatitis. Microb Pathog. 2021;154:104849.
21. van den Berg FF, van Dalen D, Hyoju SK, van Santvoort HC, Besselink MG, Wiersinga WJ, et al. Western-type diet influences mortality from necrotising pancreatitis and demonstrates a central role for butyrate. Gut. 2021;70(5):915–27.
22. Zheng J, Lou L, Fan J, Huang C, Mei Q, Wu J, et al. Commensal *Escherichia coli* aggravates acute necrotizing pancreatitis through targeting of intestinal epithelial cells. Appl Environ Microbiol. 2019;85(12):e00059–19. https://doi.org/10.1128/AEM.00059-19.
23. Zou M, Yang Z, Fan Y, Gong L, Han Z, Ji L, et al. Gut microbiota on admission as predictive biomarker for acute necrotizing pancreatitis. Front Immunol. 2022;13:988326.

24. Lei Y, Tang L, Liu S, Hu S, Wu L, Liu Y, et al. *Parabacteroides* produces acetate to alleviate heparanase-exacerbated acute pancreatitis through reducing neutrophil infiltration. Microbiome. 2021;9(1):115.
25. Prevel R, Enaud R, Orieux A, Camino A, Berger P, Boyer A, et al. Gut bacteriobiota and mycobiota are both associated with day-28 mortality among critically ill patients. Crit Care. 2022;26(1):105.
26. Li H, Xie J, Guo X, Yang G, Cai B, Liu J, et al. *Bifidobacterium* spp. and their metabolite lactate protect against acute pancreatitis via inhibition of pancreatic and systemic inflammatory responses. Gut Microbes. 2022;14(1):2127456.
27. Yan X, Li J, Wu D. The role of short-chain fatty acids in acute pancreatitis. Molecules. 2023;28(13):4985.
28. Zuza-Alves DL, Silva-Rocha WP, Chaves GM. An update on *Candida tropicalis* based on basic and clinical approaches. Front Microbiol. 2017;8:1927.
29. Di Martino L, De Salvo C, Buela KA, Hager C, Ghannoum M, Osme A, et al. *Candida tropicalis* infection modulates the gut microbiome and confers enhanced susceptibility to colitis in mice. Cell Mol Gastroenterol Hepatol. 2022;13(3):901–23.
30. Fernandez-Pacheco P, Pintado C, Briones Perez A, Arevalo-Villena M. Potential probiotic strains of *Saccharomyces* and non-*Saccharomyces*: functional and biotechnological characteristics. J Fungi. 2021;7(3):177. https://doi.org/10.3390/jof7030177.
31. Karen M, Yuksel O, Akyurek N, Ofluoglu E, Caglar K, Sahin TT, et al. Probiotic agent *Saccharomyces boulardii* reduces the incidence of lung injury in acute necrotizing pancreatitis induced rats. J Surg Res. 2010;160(1):139–44.
32. Ost KS, Round JL. Commensal fungi in intestinal health and disease. Nat Rev Gastroenterol Hepatol. 2023;20(11):723–34.
33. Schlechte J, Zucoloto AZ, Yu IL, Doig CJ, Dunbar MJ, McCoy KD, et al. Dysbiosis of a microbiota-immune metasystem in critical illness is associated with nosocomial infections. Nat Med. 2023;29(4):1017–27.
34. Xu R, Tan C, Zhu J, Zeng X, Gao X, Wu Q, et al. Dysbiosis of the intestinal microbiota in neurocritically ill patients and the risk for death. Crit Care. 2019;23(1):195.
35. Shealy NG, Yoo W, Byndloss MX. Colonization resistance: metabolic warfare as a strategy against pathogenic Enterobacteriaceae. Curr Opin Microbiol. 2021;64:82–90.
36. Park G, Munley JA, Kelly LS, Kannan KB, Mankowski RT, Sharma A, et al. Gut mycobiome dysbiosis after sepsis and trauma. Crit Care. 2024;28(1):18.
37. Mu S, Xiang H, Wang Y, Wei W, Long X, Han Y, et al. The pathogens of secondary infection in septic patients share a similar genotype to those that predominate in the gut. Crit Care. 2022;26(1):68.
38. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559–63.
39. Auchtung TA, Fofanova TY, Stewart CJ, Nash AK, Wong MC, Gesell JR, et al. Investigating colonization of the healthy adult gastrointestinal tract by fungi. mSphere. 2018;3(2):e00092–18. https://doi.org/10.1128/mSphere.00092-18.
40. Buttar J, Kon E, Lee A, Kaur G, Lunken G. Effect of diet on the gut mycobiome and potential implications in inflammatory bowel disease. Gut Microbes. 2024;16(1):2399360.
41. Zhao MQ, Fan MY, Cui MY, Chen SM, Wang JJ, Lu YY, et al. Profile of intestinal fungal microbiota in acute pancreatitis patients and healthy individuals. Gut Pathog. 2025;17(1):1.
42. Coker OO, Nakatsu G, Dai RZ, Wu WKK, Wong SH, Ng SC, et al. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. Gut. 2019;68(4):654–62.
43. Lemoinne S, Kemgang A, Ben Belkacem K, Straube M, Jegou S, Corpechot C, et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. Gut. 2020;69(1):92–102.
44. Baldassano SN, Bassett DS. Topological distortion and reorganized modular structure of gut microbial co-occurrence networks in inflammatory bowel disease. Sci Rep. 2016;6:26087.
45. Greenblum S, Turnbaugh PJ, Borenstein E. Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci U S A. 2012;109(2):594–9.
46. Frost F, Kacprowski T, Ruhlemann M, Pietzner M, Bang C, Franke A, et al. Long-term instability of the intestinal microbiome is associated with metabolic liver disease, low microbiota diversity, diabetes mellitus and impaired exocrine pancreatic function. Gut. 2021;70(3):522–30.

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