Three *Klebsiella* species as potential pathobionts generating endogenous ethanol in a clinical cohort of patients with auto-brewery syndrome: a case control study

Guanhua Xue,[a,b,h] Junxia Feng,[a,h] Rui Zhang,[a,b,h] Bing Du,[a] Ying Sun,[e] Shiyu Liu,[a] Chao Yan,[a] Xinjuan Liu,[d] Shuheng Du,[a] Yanling Feng,[a] Jinghua Cui,[a] Lin Gan,[a] Hanqing Zhao,[a] Zheng Fan,[a] Xiaohu Cui,[a] Ziying Xu,[a] Tongtong Fu,[a] Chen Li,[d] Lei Huang,[g] Ting Zhang,[b,f] Jing Wang,[d,***] Ruifu Yang,[c,**] and Jing Yuan[a,b,*]

[a]Department of Bacteriology, Capital Institute of Pediatrics, Beijing, China
[b]Children's Hospital Capital Institute of Pediatrics, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
[c]Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, China
[d]Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China
[e]The Department of Immunology, School of Basic Medical Sciences, Capital Medical University, Beijing, China
[f]Beijing Municipal Key Laboratory of Child Development and Nutriomics, Capital Institute of Pediatrics, Beijing, China
[g]Department of Infectious Diseases, The Fifth Medical Center of Chinese PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing, China

eBioMedicine
2023;91: 104560
Published Online xxx
https://doi.org/10.1016/j.ebiom.2023.104560

Summary

Background Patients with auto-brewery syndrome (ABS) become inebriated after the ingestion of an alcohol-free, high-carbohydrate diet. Our previous work has shown that high-alcohol-producing (HiAlc) *Klebsiella pneumoniae* can generate excessive endogenous ethanol and cause non-alcoholic fatty liver disease (NAFLD). Therefore, it is reasonable to speculate that such bacteria might play an important role in the pathogenesis of ABS.

Methods The characteristics and metabolites of the intestinal flora from a clinical cohort of patients with ABS were analysed during different stages of disease and compared to a group of healthy controls. An *in vitro* culture system of relevant samples was used for screening drug sensitivity and ABS-inducing factors. Rabbit intestinal and murine models were established to verify if the isolated strains could induce ABS *in vivo*.

Findings We observed intestinal dysbiosis with decreased abundance of *Firmicutes* and increased of *Proteobacteria* in patients with ABS compared with healthy controls. The abundance of the genus *Klebsiella* in *Enterobacteriaceae* was strongly associated with fluctuations of patient's blood alcohol concentration. We isolated three species of HiAlc *Klebsiella* from ABS patients, which were able to induce ABS in mice. Monosaccharide content was identified as a potential food-related inducing factor for alcohol production. Treatments with antibiotics, a complex probiotic preparation and a low-carbohydrate diet not only alleviated ABS, but also erased ABS relapse during the follow-up observation of one of the patients.

Interpretation Excessive endogenous alcohol produced by HiAlc *Klebsiella* species was an underlying cause of bacterial ABS. Combined prescription of appropriate antibiotics, complex probiotic preparation and a controlled diet could be sufficient for treatment of bacteria-caused ABS.

Funding The funders are listed in the acknowledgement.

Keywords: Auto-brewery syndrome; Gut microbiota; *Klebsiella*; Diagnosis; Treatment

*Corresponding author. Capital Institute of Pediatrics.
**Corresponding author. Beijing Institute of Microbiology and Epidemiology.
***Corresponding author. Beijing Chaoyang Hospital, Capital Medical University.
E-mail addresses: yuanjing6216@163.com (J. Yuan), ruifuyang@gmail.com (R. Yang), kyc6636@126.com (J. Wang).
[h]These authors contributed equally to this work.

Research in context

Evidence before this study

Auto-brewery syndrome (ABS) is thought to be associated with the abnormal proliferation of yeast in humans in previous studies. However, the complex gut microbiota was seldom studied on these patients, the potential pathobionts are likely to be ignored. Recently, we described a patient with severe non-alcoholic steatohepatitis accompanied by ABS, which was caused by high-alcohol-producing (HiAlc) *Klebsiella pneumoniae* through the generation of large amounts of endogenous alcohol. Anti-fungal treatments failed to alleviate the symptoms of the patient. However, the detailed pathogenesis of HiAlc *Klebsiella* and its pathobiont role in ABS progression, particularly concerning dynamic changes in gut microbiota, have not been fully elucidated.

Added value of this study

In the present study, we extensively characterized the gut microbiome of bacterial ABS in a clinical cohort, as well as the therapeutic outcome and changes in the gut microbiome of the patient. We revealed that three intestinal *Klebsiella* species including *K. pneumoniae*, *Klebsiella quasipneumoniae*, and *Klebsiella variicola* could produce higher alcohol and were the causative agent of these bacterial ABS. We made a therapeutic regimen involving antibiotics, complex probiotic preparation, and restricted carbohydrate intake, which alleviated ABS and prevented further episodes. All these findings augmented our current knowledge and provided a new way for diagnosis and treatment of ABS.

Implications of all the available evidence

The findings described in the present study shed light on the pathogenesis of ABS. The overgrowth of some endogenous alcohol production bacteria in intestinal was linked to the occurrence of the disease. Furthermore, the endogenous ethanol production is importance not only in ABS but also non-alcoholic steatohepatitis (NASH) and perhaps many other ethanol-relevant diseases.

Introduction

Auto-brewery syndrome (ABS), also known as gut fermentation syndrome, is a condition in which patients become inebriated after the ingestion of an alcohol-free, high-carbohydrate diet.[1,2] The earliest cases of ABS were reported in France in 1894 and translated from French into English in 1906 in the book Auto-Intoxication in Disease.[3] More patients with ABS have since been described, in whom the causative organisms have mostly included *Candida* spp. and *Saccharomyces* spp. (*Candida albicans, Candida krusei, Candida glabrata, Candida intermedia, Candida parapsilosis, Candida kefyr*, and *Saccharomyces cerevisiae*),[4–10] the authors of these reports have assumed that the causative organisms could ferment carbohydrates into excess alcohol in the gut, thus producing a state of inebriation.

Normally, small amounts of endogenous ethanol are formed inside cells as metabolic intermediaries or products. Under anaerobic conditions in the colon, carbohydrate-derived pyruvate can be metabolized into acetaldehyde, which is then reduced to form ethanol.[11,12] This process is favoured during intestinal overgrowth of bacteria or yeast and during excessive consumption of carbohydrates.[13] The resulting endogenous ethanol is rapidly and almost completely removed from the blood by intestine and liver alcohol dehydrogenases (Adh), catalases, and the microsomal ethanol-oxidizing system. However, when the endogenous ethanol exceeds the metabolic capacity of the intestine and liver, it will accumulate in the body, resulting in elevated blood alcohol concentration (BAC) and the onset of ABS.

Previous reports have indicated that ABS can be caused by the abnormal proliferation of yeast.[4–10] However, these studies were mostly based on faecal testing of yeast growth and empiric therapy. In fact, considering the complex gut microbiota, the potential pathobionts are likely to be ignored. It is well known that intestinal dysbacteriosis is associated with various chronic diseases,[14–17] and the overgrowth of some bacteria with the ability of generating alcohol in the intestine may also play an important role in ABS. Recently, we have described a patient with severe non-alcoholic steatohepatitis (NASH) accompanied by ABS caused by high-alcohol-producing (HiAlc) *K. pneumoniae* through the generation of large amounts of endogenous alcohol, and anti-fungal treatments had no effects on the patient's symptoms.[18] Thus, it is reasonable to propose that HiAlc *K. pneumoniae* is a causative agent of bacterial ABS. However, the detailed pathogenesis of HiAlc *K. pneumoniae* and its pathobiont role in ABS progression, particularly concerning dynamic changes in gut microbiota, has not been fully elucidated. Our previous findings indicate a need for further evidence to support the role of HiAlc *Klebsiella* in ABS progression in a cohort, which may guide clinical treatment.

During the past 3 decades, most ABS cases were reported as anecdotes.[1–4] To our knowledge, there are not clinical consensus guidelines for the gut microbiota changes, and treatment of bacterial ABS. Most patients are treated with a controlled diet or empirical antifungal therapy.[4–6,8–10,19,20] Despite the need for evidence-based treatment of ABS, variations in gut flora among affected patients have not been fully characterized. Based on the previous studies, we speculate that some bacteria, such as *Klebsiella*, play an important role in the pathogenesis of ABS. These overgrown bacteria in the gut of ABS patients can produce large amounts of

endogenous ethanol, leading to the patient's state of intoxication. Targeted use of antibiotics for microbiota can effectively control the clinical symptoms of ABS patients who fail to respond to fungal treatment. To verify this hypothesis, we conducted the present study in a clinical cohort.

Methods

Study design and participants

The study design is a case control with a group of patients with ABS and a group of healthy controls. ABS patients were recruited at Beijing Chaoyang Hospital and Capital Institute of Pediatrics, China from May 2020 to August 2022. Seven patients with history of repeated episodes of unexplained intoxication without the intake of alcohol products presented to our clinic for a confirmatory diagnosis and subsequent treatment. All patients had a history of symptoms of ABS, becoming inebriated after the ingestion of an alcohol-free, high-carbohydrate diet. Concentrations of blood alcohol of all patients were tested at one, two, four and 6 h after challenging with 75 g glucose. Five of the seven patients who had elevated BAC were enrolled in the present study for further investigation, numbered as ABS-1 to ABS-5 according to the time of enrollment (Fig. S1). All patients were being followed up.

Participants for the healthy control (HC) group (n = 5) were recruited from staff working at Capital Institute of Pediatrics and staff's family, considering the matched age (range: ±2 years), sex and BMI range (±1) of the enrolled ABS cases. These control subjects did not have any unexplained intoxication symptoms or other medical conditions, especially gastrointestinal disease and nervous system disease. The code for HCs were numbered as HC1 to HC5 according to the matching age, sex and BMI at enrollment.

Ethics approval and consent to participants

The study was performed in compliance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) and was approved by the Research Board of the Ethics Committee of the Capital Institute of Pediatrics and Beijing Chaoyang Hospital (Number 2021320). All participants signed an informed consent form prior to entering the study. The animal experiment was approved by the Research Board of the Ethics Committee of Capital Institute of Pediatrics (Number DWLL2021005).

Faecal sampling

A total of 62 fresh faecal samples from these ABS patients were collected at their stable, pre-onset, onset, and recovery stages of clinical progression for subsequent sequencing. Based on the clinical symptoms of each patient, five, thirty-seven, four, five, and eleven faecal samples for ABS-1, ABS-2, ABS-3, ABS-4, ABS-5 were collected separately. Faecal samples from five healthy individuals were collected three times each to exclude sampling error (Table S1). To exclude the influence of other factors, no medications and probiotics were taken during the first 2 weeks of sampling.

In vitro studies for faecal samples

Metagenome sequencing and metabolomics of faecal samples: Metagenome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China), in accordance with previously published methods.[21–25] Untargeted metabolomics profiling was performed using the XploreMET platform (Metabo-Profile, Shanghai, China). Sample preparation was performed using previously described methods with minor modifications.[26,27]

Meta-fermentation of faecal microbiota: Faecal samples from all participants were pre-processed, then subjected to fermentation using an *in vitro* culture system in Yeast Extract Peptone Dextrose (YPD) medium; the system contained a range of carbohydrates or proteins as the sole carbon source for fermentation (e.g., glucose, fructose, galactose, mannose, maltose, dextrin, casein, starch, or mucin). Ethanol concentrations were measured at 8-h intervals by headspace gas chromatography (Agilent 6850) with flame ionization detection (Headspace).[28]

Detection of copy numbers of *Klebsiella* in faecal samples: The copy numbers of *Klebsiella* in faecal samples were determined with digital polymerase chain reaction (PCR) and a TD-1 Droplet Digital PCR system (TargetingOne, Beijing, China) in accordance with the manufacturer's instructions.[29] The primers and probes used for ddPCR reactions were designed according to the citrate synthase gene, *gltA*. The details of above methods are in the Supplemental Material.

In vivo assessment of alcohol-producing abilities and construction of ABS mouse model

Rabbit intestinal culture model: An *in vivo* assay of the isolated alcohol-producing strains using a rabbit intestinal culture model was performed as previously described.[30] The alcohol concentrations were measured *in vivo*, *in vitro*, and in colonic mucus. The assay was performed at least three times using samples from all eight rabbits.

Construction of murine model of ABS induced by HiAlc *Klebsiella*: Specific-pathogen-free male C57BL/6J mice (6–8 weeks old) were fed with normal chow diet and autoclaved tap water ad libitum for 5 days, then randomly divided into eight groups (16 in each group). Groups included one ethanol-fed (EtOH-fed, positive control) group, three bacterial alone-fed groups, three bacterial with fructose-fed groups and one YPD-fed medium (pair-fed, negative control) group.

Mice in the bacterial alone groups were gavaged with 150 μL of the isolated HiAlc *Klebsiella* bacterial (K1, K2 and K3) solution (~10^8 colony-forming units) once daily for 7 days. For the bacterial with fructose groups, except for bacterial gavage, mice were concurrently provided 30% fructose solution instead of water. On day 8, mice of the above six groups were gavaged with 150 μL of HiAlc *Klebsiella*. In the positive control group, EtOH-fed mice were gavaged with 40% ethanol alone, while pair-fed mice were gavaged with YPD medium alone for 8 days. The blood samples of each group were collected at 0, 1, 2, 3 and 4 h after the last treatment. BAC of each mouse was measured via headspace gas chromatography.

Clinical verification of HiAlc *Klebsiella* contributions via microbiome-guided selection of antibiotics treatment

In vitro antibiotics sensitivity on the production of ethanol and short chain fatty acids: The effects of various antibiotics on the production of ethanol and short chain fatty acids were tested using an *in vitro* culture system. Briefly, fresh faecal samples of patient ABS-2 and five healthy controls were collected and inoculated into a series of YPD glucose medium preparations, where each preparation was supplemented with one of the following antibiotics: cefixime (1.0 mg/L and 4.0 mg/L), imipenem (4.0 mg/L and 16.0 mg/L), metronidazole (1.0 mg/L and 4.0 mg/L), levofloxacin (1.25 mg/L and 2.5 mg/L), and vancomycin (4 mg/L and 32 mg/L). Concentrations of ethanol and short chain fatty acids (e.g., acetate acid, propanoic acid, butanoic acid, and pentanoic acid) were assessed at 8-h intervals.

Clinical verification of HiAlc *Klebsiella* contributions: Faecal samples from ABS-2 were collected during the antibiotic and probiotic treatment period for analysing the gut microbiota by metagenome sequencing. The BAC and abundance of *Klebsiella* spp. in the patient were monitored throughout the treatment period.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (IBM Corp., USA). Data are expressed as means ± standard deviations (SD). Bray–Curtis dissimilarity index was performed to detect the significant difference in bacterial composition between ABS samples and healthy controls. The Kruskal–Wallis test was employed to analyse the statistical significance of the different taxonomic (phylum, family, genus) levels. The relative abundance of these features was subjected to statistical analyses. Spearman's rank coefficients were used to test for correlation of bacterial abundance at family or genus level with BAC and correlation among different genera. Linear discriminant analysis (LDA) and effect size (LEfSe) analysis were used to determine the organisms most likely to explain differences between the ABS and healthy control group. Different features with an LDA score cut-off of 2.5 were identified. Student's *t*-test (comparisons between two groups) or one-way analysis of ANOVA (comparison among ≥3 groups) was used for group comparisons. *P*-values <0.05 were considered statistically significant.

Role of funders

The funders had no role in study design, data collection, analyses, interpretation or writing of reports.

Results

Participants and clinical characteristics

From May 2020 to August 2022, seven patients were identified. Five of them met the criteria for enrollment in the present study for further investigation. All five patients were male, had a penchant for a high sugary diet, and had the clinical features of repeated episodes of unexplained alcohol intoxication without the intake of alcohol products. The symptoms were exacerbated by carbohydrate intake. Fifteen healthy controls were initially recruited in this study and ten of them were excluded due to unmatched age, sex and BMI.

Various symptom-related tests were performed on the 5 ABS patients, including abdominal computed tomography (CT), chest CT, esophagogastroduodenoscopy and colonoscopy, as well as neurologic and psychiatric assessments. Results were negative except for fatty liver disease. For patients ABS-1, ABS-2, and ABS-5, antifungal drugs had been used for one month or more prior to sampling.

In addition, the alcohol dehydrogenase and aldehyde dehydrogenase levels of these five patients were all within normal ranges. Whole-exome sequencing of blood samples did not show any relevant mutations, insertions, or deletions in *adh* and *aldh* genes. Characteristics of the ABS patients and the HCs are summarized in Table 1. Patients ABS-2 and ABS-5 with a severe onset of inebriation (BAC 40–90 mmol/L), experienced frequent hospitalizations. The BAC of the other three patients (BAC 18–44 mmol/L) could be reduced through strict diet control.

Alterations of gut microbiota and metabolites in ABS patients

To explore potential causative agents for these five patients' symptoms, stool and urine samples were first collected for fungal culture and internal transcribed spacer rDNA PCR detection. Only two types of fungi, *Candida guilliermondii* and *Rhodotorul amucilaginosa*, with very low abundance were isolated from ABS-3 at the stage of pre-onset, while no fungi were isolated from the other 4 patients. The growth rates of these two strains were slow and their respective alcohol production abilities were 7.2 mmol/L and 5.9 mmol/L after 48 h of culture, suggesting these fungi might not be the real cause of ABS.

Characteristic	Patients					Healthy control				
	ABS-1	ABS-2	ABS-3	ABS-4	ABS-5	HC-1	HC-2	HC-3	HC-4	HC-5
Onset BAC[a] (mmol/L)	18–44	40–90	18–44	18–44	40–90	–	–	–	–	–
Age	38	63	36	35	67	38	62	38	33	68
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Weight (kg)	65	62	85	70	70.8	80	65	66.5	79.8	68
BMI	22.1	19.8	27.2	26	23.4	27.1	20.8	23	25.2	22.7
Psychiatric history	No	No	No	No	No	No	No	No	No	No
Fatty liver disease	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Diabetes	No	No	No	No	No	No	No	No	No	No
Allergy to drugs/food	No	No	No	No	No	No	No	No	No	No
Surgery	No	Yes[b]	No	No	No	No	No	No	No	No
Antifungal drug use	Yes[c]	Yes[c]	–	–	Yes[c]	–	–	–	–	–
ALT (U/L)	29.3	65.5	15.2	113	26.2	19	18	19	13	17
AST (U/L)	26.4	91.7	18.5	120	24,5	11	18	15	20	14

Patients were numbered as ABS-1 to ABS-5. Healthy controls were numbered as HC1 to HC5 according to the matching age, sex and BMI of ABS patients. ALT: Alanine transaminase; AST: Aspartate aminotransferase. [a]Blood alcohol concentration at the onset stage. [b]Pancreatic cystectomy 2019-05. [c]Before faecal sampling, ABS 1 had taken Fluconazole (PO) for 1.5 month. ABS 2 had used Fluconazole (PO) for 1 month; and ABS 5 had used Nystatin for 1 month.

Table 1: **The clinical characteristics of five ABS patients and five healthy controls.**

Metagenomic analysis was performed in faecal samples collected from the onset stages of the five ABS patients and five healthy controls. An average of 1,414,361 ORF contigs in non-redundant gene catalog was obtained in each sample, which was mainly dominated by the phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria*. Primarily, principal coordinate analysis (PCoA) based on the Bray–Curtis dissimilarity index showed the bacterial composition difference between ABS samples and HCs (ANOSIM test, $p = 0.007$, Fig. 1a). Data indicate there was significant intestinal dysbiosis in the ABS cohort, which was characterized by increased relative abundance of *Proteobacteria* and decreased *Firmicutes*, compared with samples from HCs. To further identify the main microbial taxa contributing to the onset of ABS, differential enrichment taxa were analysed, which led to *Gammaproteobacteria*, *Enterobacterales*, and *Enterobacteriaceae*. Furthermore, most of the increased *Proteobacteria* were dominated by the fifteen abundant families, of which, *Enterobacteriaceae* had the highest abundance and showed a significant difference between the ABS and HC group (Kruskal–Wallis test, $p < 0.05$) (Fig. 1b). Notably, the average BAC level of each patient and the abundance of *Enterobacteriaceae* strongly correlated with each other (Fig. 1c). Statistically, when compared with HC group, some genera in *Enterobacteriaceae* were enriched in the ABS group, including *Escherichia*, *Klebsiella*, *Shigella*, *Salmonella*, *Citrobacter*, *Enterobacter* and *Raoultella*, which indicated their potential pathobionts' role to ABS onset (Kruskal–Wallis test, $p < 0.05$) (Fig. 1d). The cladogram also indicates the specific bacteria associated with ABS patients (Fig. 1e).

Furthermore, we quantified 284 metabolites in these ABS patients, and the abundances of 77 metabolites differed between ABS samples and HC samples (Fig. S2a). Intriguingly, multiple neurotransmitters and specific metabolites (e.g., gamma-aminobutyric acid, dopa, 5-hydroxydopamine, 2,3-butanediol, chenodeoxycholic acid, tyrosine, phenylalanine, benzyl alcohol, tryptophan, and putrescine) significantly increased during the onset of an ABS episode. In contrast, the abundances of serotonin and lithocholic acid decreased (Fig. S2b and c). These results indicate the potential for neurotransmitter-mediated changes in the patient's mood via the gut–brain axis, similar to the neural signalling that occurs during alcohol intoxication.

The dynamic changes and correlation of gut microbiota and BAC

Because patient ABS-2 with a severe onset of inebriation experienced frequent hospitalizations, his samples were collected at different periods, especially during treatment to elaborate the dynamic changes of microbiota and BAC. From November 16 to December 20 in 2020, continuous blood alcohol monitoring and metagenomic analyses of faecal samples were conducted daily in patient ABS-2 who experienced five episodes (lasting for 3–5 days per episode). His BAC reached 300–400 mg/dL (65 mmol/L–87 mmol/L, equivalent to 15 shots of whisky with 40% alcohol by volume) during each episode. After interventions including a controlled diet and probiotics, the patient experienced temporary improvement and began a long period of recovery (Fig. 2a).

Comparisons of metagenomic data with BAC data of ABS-2 revealed that the distribution (by percentage) of the *Enterobacteriaceae* was strongly correlated with BAC fluctuation (Fig. 2b), which was consistent with the

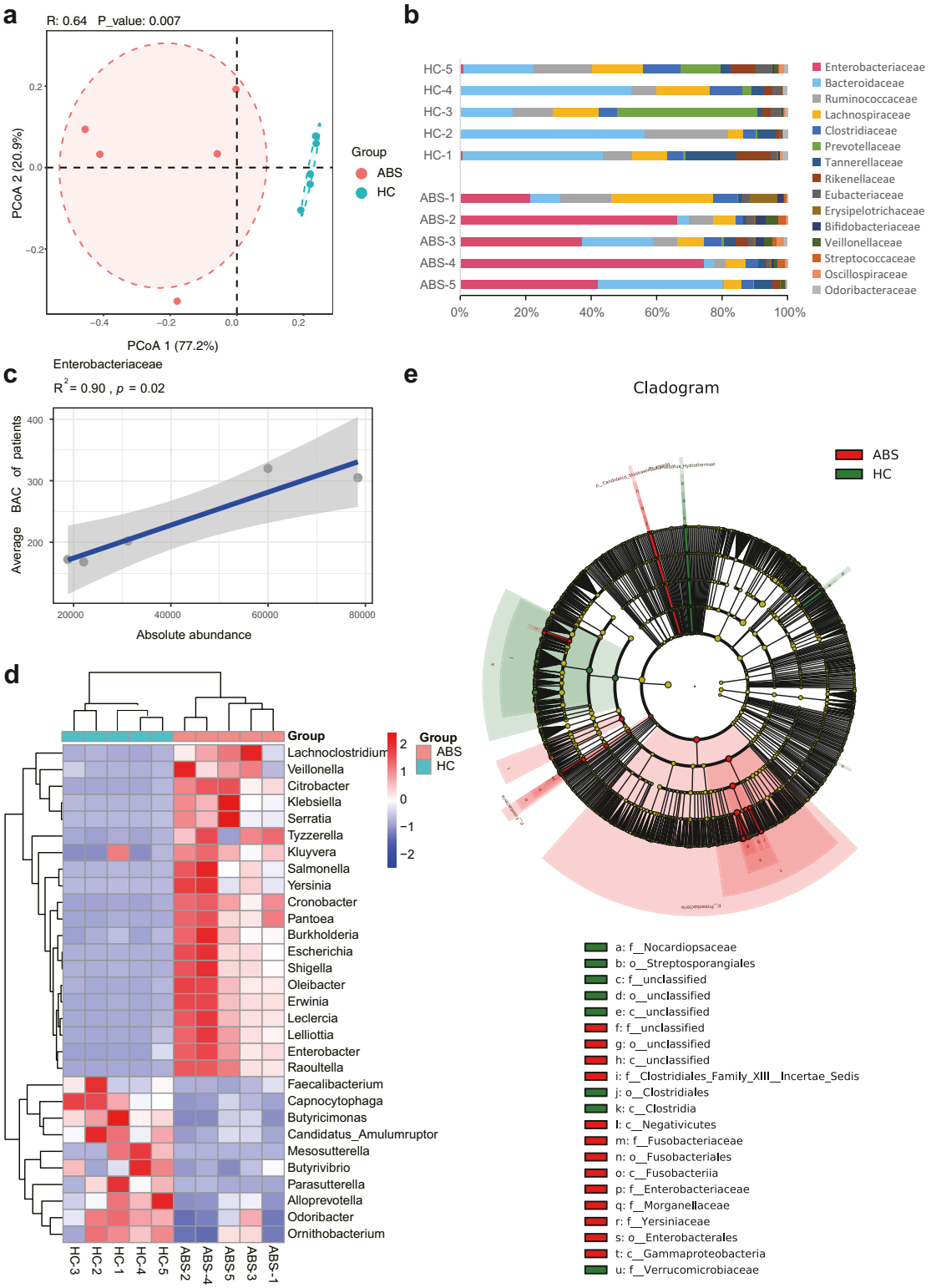

Fig. 1: **Gut microbiome characterization and bacterial diversity of ABS patients. (a)** PCoA based on the Bray–Curtis dissimilarity index shows the between-subjects (β) diversity across groups, in which the blue and red dot represent HC and ABS subjects, respectively. (ANOSIM, p = 0.007) **(b)** The top fifteen abundant families of intestinal microbiota community between HC and ABS subjects shows the intestinal

cohort study results. Furthermore, the genus *Klebsiella*, *Escherichia*, *Shigella*, *Salmonella* and *Enterobacter* showed a higher absolute abundance and were positively correlated with the change of BAC (Fig. 1b). Interestingly, the abundance variation trend of the five genera were significantly correlated with BAC fluctuation (Spearman's rho were 0.89, 0.85, 0.85, 0.85 and 0.84, respectively), which increased during the onset states (S1, S2, S3, S5, S6, S12, S14, S16, S17, and S18). When the BAC reached 400 mg/dL (87 mmol/L) during onset state (S2), it was almost 60-fold greater than in pre-onset samples or samples from healthy controls (Fig. 2c). Notably, we found positive correlation between these five genera in *Proteobacteria* (Fig. 2d), possibly explaining that all genera had the same tendency. Also, it was consistent with the same results in other patients' samples (data not shown). These findings further suggest that *Proteobacteria* and *Enterobacteriaceae* are strongly associated with ABS.

To further investigate which genus contributed for the ABS onset, faecal samples obtained from all five ABS patients were cultured with YPD medium. A total of 102 bacteria were isolated and the alcohol production abilities of these bacteria in each different genus were tested. The results showed that genus *Klebsiella* including *K. pneumoniae*, *K. quasipneumoniae* and *K. variicola* produced the greatest amounts of alcohol when compared with other genera (Fig. 2e, Table S2), implying that *Klebsiella* may be the potential pathobiont of ABS.

HiAlc *Klebsiella* contributed to ABS onset through the endogenous production of alcohol

To understand further HiAlc *Klebsiella* contribution, gastrointestinal lavage fluid of patient ABS-2 was collected via upper and lower endoscopy during hospitalization. Alcohol concentrations at different intestinal sites were detected via fermentation culture. Notably, high concentrations of alcohol (>10 mmol/L) were detected in intestinal lavage fluid, along with the presence of *Klebsiella* and the abundance of *Klebsiella* spp. was higher at sites with high concentrations of alcohol (Fig. 3a–c). These findings further support that *Klebsiella* could be as an underlying cause of ABS.

We also isolated 17 strains of HiAlc *K. pneumoniae*, *K. quasipneumoniae* and *K. variicola* from the small intestine, colon, and faeces of ABS-2. Then, we selected the three above species with the greatest alcohol producing ability under both aerobic and anaerobic conditions for physiological characteristics analysis, named as HiAlc *K. pneumoniae* K-1, *K. quasipneumoniae* K-2 and *K. variicola* K-3. These HiAlc *Klebsiella* strains constituted typical mucoid lactose fermenters, and had clear capsules and biofilms, along with higher growth speed (Fig. 3d). Cultivation experiments revealed that alcohol production abilities of these strains were related to the carbon source (e.g., monosaccharide) and environmental oxygen conditions (Fig. 3e), but there was no significant difference between glucose and fructose alcohol production (t-test, $p > 0.05$).

HiAlc *Klebsiella* produced alcohol *in vivo* and induced murine model of ABS

To confirm that HiAlc *Klebsiella* can also produce alcohol *in vivo*, a rabbit intestinal culture model was established. The results showed that alcohol concentrations were comparable among *in vivo* (~30 mmol/L), colonic mucus (~20 mmol/L), and *in vitro* (~40 mmol/L) samples, 4 h after each of the strains had been cultured. All three HiAlc *Klebsiella* strains had a strong alcohol-producing ability *in vivo*, compared with the ability of standard strain ATCC 2146 (Fig. S4a and b).

To confirm that HiAlc *Klebsiella* could induce ABS, we constructed a mouse model of 8 days of gavage with the bacteria and a fructose solution. Compared with the pair-fed group, the BAC in HiAlc *Klebsiella* plus fructose-fed mice increased at 3–4 h after the last gavage and then gradually decreased. Furthermore, the mice showed similar symptoms of intoxication to the EtOH-fed group. These results suggest that all three *Klebsiella* species could produce alcohol by fermentation in the intestinal tract, which was sufficient to induce ABS in the experimental mice (Fig. S4c and d). Taken together, these data show that HiAlc *Klebsiella* produced alcohol *in vivo*, induced ABS and might be a pathobiont in ABS development.

Identification of dietary components that induce alcohol production by HiAlc *Klebsiella*

To investigate the effects of dietary components inducing alcohol production, faecal samples from the onset and recovery stage of the five ABS patients were cultured in media contained carbohydrates or proteins at various concentrations, while corresponding samples from the five healthy individuals were used as controls. As shown in Fig. S3, up to 35 mmol/L of alcohol could be generated by faecal microbiota that were collected from onset stage of ABS patients and cultured in media

dysbiosis based on Kruskal–Wallis test. **(c)** The Spearman's correlations analysis showing that correlation of average BAC level of each patient and abundance of *Enterobacteriaceae*. **(d)** Heatmap shows the abundance of 30 difference taxa in genus enriched in HC and ABS group (Kruskal–Wallis test). **(e)** A cladogram to identify the specific bacteria associated with ABS patients (Linear discriminant analysis with cutoff value 2.5). Red nodes indicate microbial taxa that play an important role in ABS group, green nodes indicate microbial taxa that play an important role in HC group, and yellow nodes indicate no difference taxa.

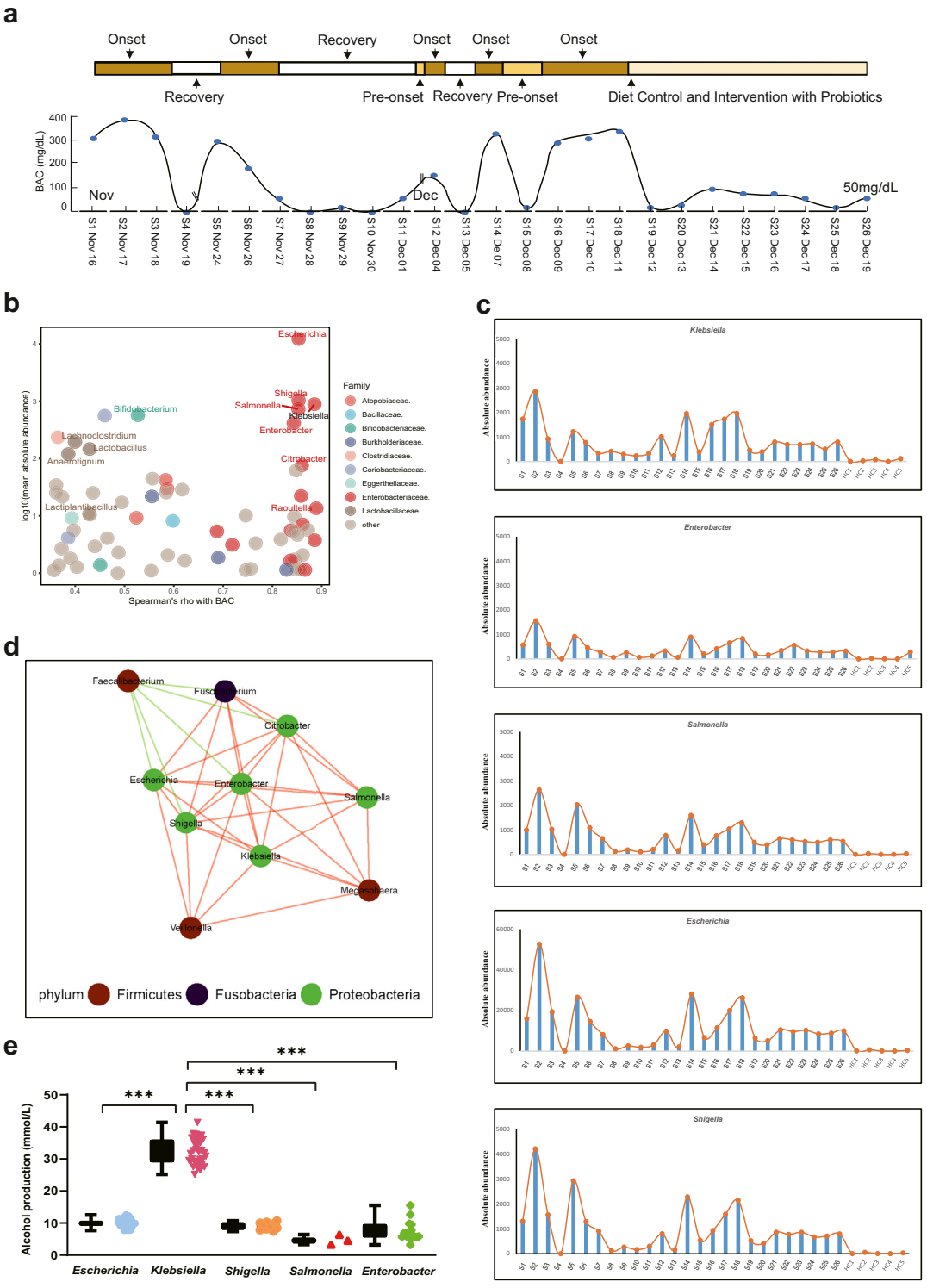

Fig. 2: **Correlations of intestinal microbiota and blood alcohol concentration of patient. (a)** Timeline of observation and treatment for the patient ABS 2. The stool and blood samples of patient ABS-2 were collected during different stages and marked as from S1 to S26 (1 mmol/L = 4.6 mg/dL). **(b)** The correlation between different genus abundances and blood alcohol concentration (BAC) was measured by Spearman's rank correlation coefficient. **(c)** The variation abundance of different genus and BAC fluctuation during the entire monitoring period from November 16, 2020 to December 19, 2020. **(d)** Microbial network showed strong correlation among different genus in *Proteobacteria*. **(e)** The

containing different monosaccharides. In contrast, the concentrations of alcohol were much low (<10 mmol/L) in the same bacteria cultured with media containing other substrates. Correspondingly, concentrations of alcohol were also low in cultures of faecal microbiota collected from the recovery stage of ABS patients or from healthy controls. These results indicated that monosaccharides could be effectively fermented into alcohol by HiAlc *Klebsiella*.

Clinical verification of *Klebsiella* species as potential pathobionts via microbiome-guided selection of antibiotics treatment

To guide establishment of a treatment strategy, we further took patient ABS-2 as an example to analyse the intestinal microbiome sensitivities to various antibiotics. Importantly, the usage of levofloxacin and imipenem significantly reduced the alcohol-producing abilities of intestinal microbiota. Additionally, high-performance liquid chromatography revealed that short chain fatty acids (e.g., acetate acid, propanoic acid, butanoic acid, and pentatonic acid), which are the main by-products of fermentation, were also significantly reduced in the presence of levofloxacin and imipenem (Fig. 4a). Faecal fermentation samples in the presence and absence of antibiotics were collected for the identification of relevant microbiota. Importantly, *Klebsiella* abundance was significantly reduced in the presence of levofloxacin and imipenem, consistent with the drug-resistance spectrum of HiAlc *Klebsiella* isolates obtained in this study. These findings strongly suggest that HiAlc *Klebsiella* is the causative organism in patient ABS-2.

Based on the above findings, microbiota related treatment was conducted on patient ABS-2. After supplementation with a 15% amino acid and vitamin C injection (250 mL/day) for 3 days (March 29–31, 2021; S28), the patient was restricted to a sugar- and carbohydrate-free diet combined with oral antibiotic (levofloxacin 500 mg/day) treatment in the hospital for 1 week (April 1–7, 2021; S29–S33). During this period, his symptoms of intoxication gradually subsided. Subsequently, gut microbiota reconstitution was performed using commercial probiotics, on the basis of differences in intestinal microbiota between the patient and healthy controls. A commercial complex probiotic that contained 26 types of *Lactobacillus* (twice daily) and a probiotic containing *Clostridium butyricum* and *Bacillus* (once daily) were used for 10 days; subsequent probiotic administration comprised the commercial *Lactobacillus* complex for 5 days (Fig. 4b).

Throughout the treatment process, the patient's BAC continuously fluctuated from 0 to <13 mmol/L, and finally decreased to 0 mmol/L on April 7, 2021. At that time, both the copy numbers of *Klebsiella* in faecal samples and the patient's BAC also significantly decreased (Fig. 4c). In this case, this patient was discharged from the hospital. Additionally, we analysed the dynamic changes in the patient's gut microbiota before and after treatment, which revealed the effects of treatment. Results showed that the abundance of *Klebsiella* spp. (red bar) was consistent with the patient's clinical course (Fig. 4d), providing further support for HiAlc *Klebsiella* as the causative gut microbe. Also, it was consistent with the same results in other patients (data not shown). We have conducted monthly follow-up since the patients was discharged, revealing that the patient's symptoms have not recurred.

Discussion

Although the incidence of ABS is relatively rare, patients with this syndrome carry many of the medical and social implications of alcoholism.[31,32] Previous reports have indicated that ABS might be caused by the abnormal proliferation of yeast in the gut, urinary tract and oral cavity.[33–35] Thus, yeast has long been regarded as the culprit of ABS. In our previous study, we have isolated HiAlc *K. pneumoniae* from an ABS patient, who did not respond to anti-fungal treatments.[18] Based on the discovery of this case and our data, it is reasonable to further speculate that some bacteria might contribute to the onset of ABS or the patients might have concurrent bacteria with fungi. Therefore, we proposed the concept of bacterial ABS. However, because the causal relationship between changes in the gut microbiome and ABS remains unclear, detailed information is needed to fully elucidate the causative gut microbes, such as HiAlc *Klebsiella*, as a contributor to the pathogenesis of ABS. Furthermore, patients with ABS worldwide have no effective treatment options, which forces these patients to form the Auto-Brewery Support Group (with over 700 members) to seek help. Despite the need for evidence-based treatment of ABS, variations in gut flora among affected patients have not been fully characterized. Therefore, a cohort of ABS patients is important to explore the disease etiology and treatment more fully, as well as the gut microbiota in affected patients.

In the present study, we extensively characterized the gut microbiome of bacterial ABS in a clinical cohort, as well as the therapeutic outcome and changes in the gut microbiome of one patient. It was noted that enteric

alcohol-producing ability of the isolated bacterial in different genus of *Enterobacteriaceae*, including *Klebsiella* (n = 35), *Escherichia* (n = 39), *Shigella* (n = 10), *Salmonella* (n = 3) and *Enterobacter* (n = 15). Each genus is presented with means ± standard deviations (left) and scatter plot (right). Blue dot for *Escherichia*, pink triangle for *Klebsiella*, yellow dot for *Shigella*, red triangle for *Salmonella*, and green square for *Enterobacter*.

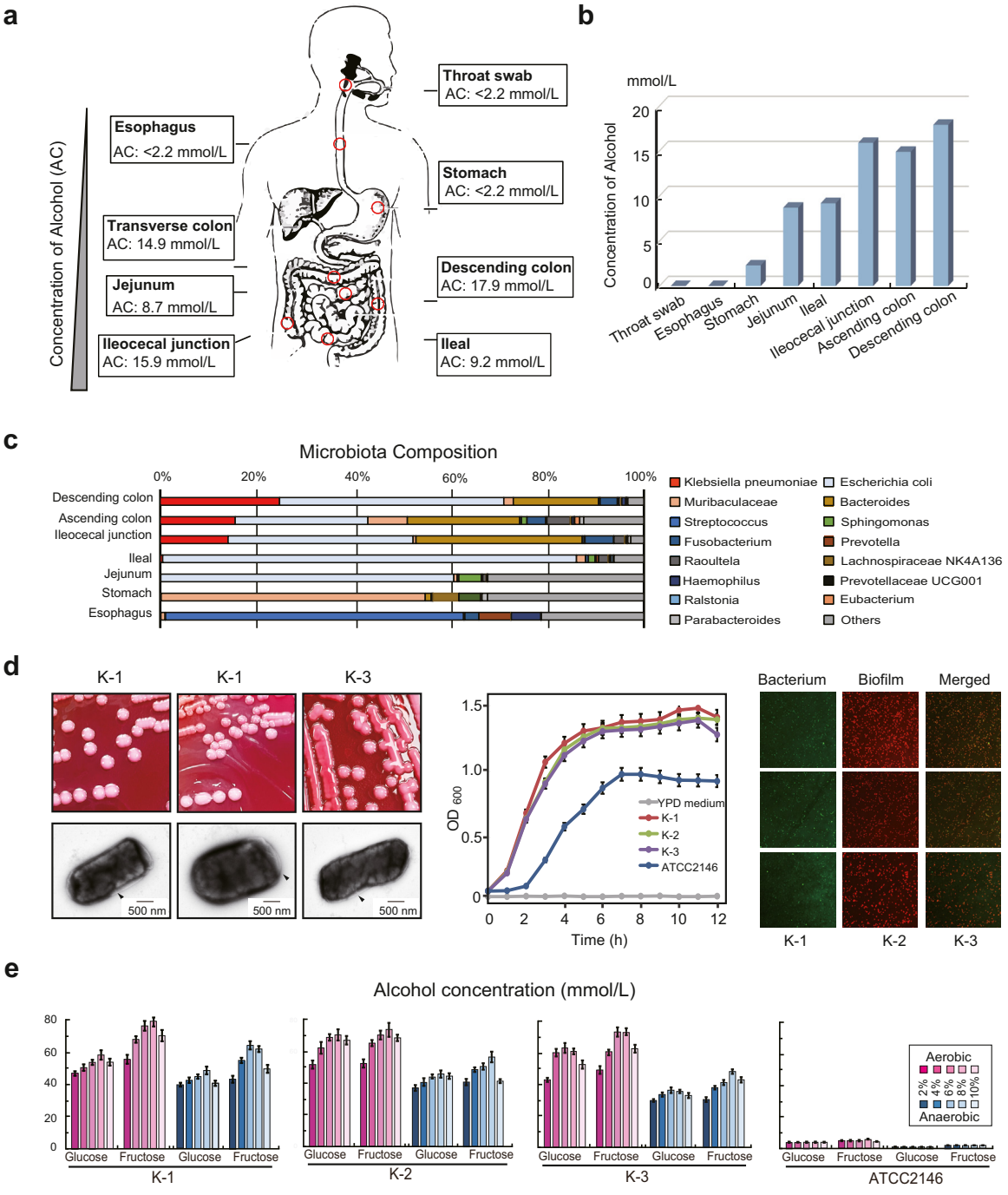

***Fig.* 3: Alcohol producing capacity of gastrointestinal lavage fluid from different parts of ABS patients under esophagogastroduodenoscopy (EGD) and colonoscopy. And characteristics of HiAlc *Klebsiella* isolated from ABS patients. (a)** Gastrointestinal lavage fluid from different parts of ABS-2 were collected under EGD and colonoscopy. Concentration of alcohol (AC) in these parts of patient ABS-2 were tested by headspace gas chromatography method. Bacterial in these parts were also isolated and the alcohol producing capacity were tested. **(b)** Concentration of alcohol from different parts of patient ABS-2. **(c)** Intestinal microbiota community from different parts of patient ABS-2. **(d)** Images of typical colonies and capsules (marked with arrows), growth curves, images of biofilm formation of the three HiAlc *Klebsiella* isolates (*K. pneumoniae*, *K. quasi pneumoniae*, *K. variicola* named as K-1, K-2, and K-3). **(e)** Ethanol producing capacity (alcohol concentration Y-axis) in different concentrations in glucose and fructose under aerobic (different shades of red) and anaerobic (different shades of blue) conditions.

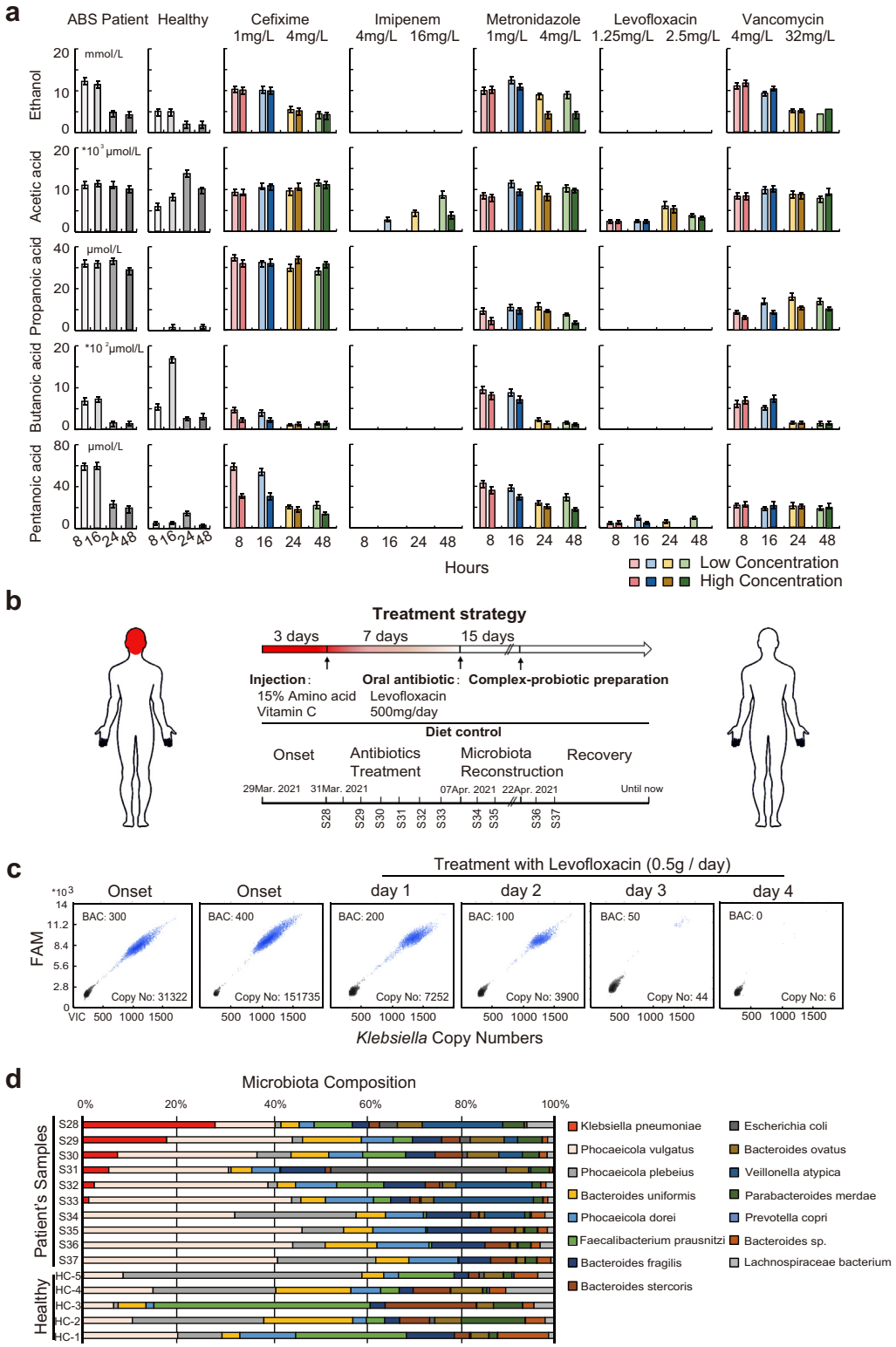

Fig. 4: **Effect of different antibiotics on intestinal microbiota. (a)** Capabilities of alcohol-producing (Panel 1) as well as short chain fatty acid-producing intestinal microbiota under various antibiotic stresses in an *in vitro* microbiota fermentation system. Acetate (Panel 2), propanoic (Panel 3), butanoic (Panel 4), and pentanoic acid (Panel 5) are shown. Two concentrations (low and high) of different antibiotics were used

dysbiosis was observed in the five ABS patients, with the abundance of *Firmicutes* decreased and *Proteobacteria* increased, when compared with healthy controls. The dynamic correlation analysis with alcohol revealed that 5 genera in the family-*Enterobacteriaceae* were closely related to the pathogenesis of ABS, which all had an ethanol producing ability but *Klebsiella* showed the highest. These data support our hypothesis that many ethanol-producing bacteria in the gut, especially *Klebsiella* are involved in the etiology of ABS. These findings are consistent with our previous study.[18] Furthermore, some other studies have reported that *Escherichia* can produce endogenous ethanol, leading to the elevated BAC and occurrence of NASH and other diseases.[36] Taken together, all these findings implicate that the endogenous ethanol produced by these bacteria might be related to the pathogenesis of many diseases.[37] In addition, it has been shown that *Lactobacillus* can also ferment carbohydrates to alcohol in human gut and has been implicated in ABS patients in previous studies.[38] In the present study, we did not observe significant correlation of *Lactobacillus* (Spearman's rho 0.43) with BAC when compared with *Klebsiella* (Spearman's rho 0.89, Fig. 2b), or significant difference of *Lactobacillus* abundance between our ABS cohort and HCs. The contribution of this species need to be further studied.

Interestingly, there was a positive correlation between the variation of the five genera of *Enterobacteriaceae*. This possibly explained why all these bacteria had the same variation trends with patients' alcohol levels, although the mechanism of interaction among these genera remains to be further explored. In addition, we identified three different HiAlc *Klebsiella* species from ABS patients, which also produced high concentrations of alcohol *in vivo*, along with the induction of ABS symptoms in mice. These findings broaden our understanding of the etiology of ABS.

To our knowledge, a few cases of ABS have been reported during the past three decades and most have been anecdotal, indicating that ABS is a very rare medical condition. Thus, treatments have generally been empirically administered with antifungal agents.[4–6,8–10,19,20] Although a few ABS studies presented treatment strategy with probiotics combined with antifungal drugs,[9] dynamic changes in gut microbiota (e.g., before and after treatment) have not been extensively analysed. To further explore this, we analysed changes of the gut microbiota and food inducing factors of five patients during their different clinical courses. Furthermore, by using an *in vitro* culture system, we collected samples from a patient (ABS-2) with a severe onset of inebriation at different periods for drug sensitivity screening and verifying the contribution of *Klebsiella* species as potential pathobionts via microbiome-guided selection of antibiotic treatment. Based on the data, we established a treatment regimen containing a carbohydrate-free diet combined with oral levofloxacin treatment for 1 week, followed by administration of a complex probiotic preparation. Our data show that such an approach effectively reduced the frequency and severity of ABS episodes, leading to recovery that has persisted for nearly 1 year. Additionally, we found that the abundance of *Klebsiella* spp. was consistent with the treatment effects and the patient's symptoms.

Notably, *K. pneumoniae* has previously been identified in some patients with ABS, but its role was neglected because of concurrent yeast detection despite the greater alcohol-producing ability of *K. pneumoniae*.[9,35] Some yeasts are known to ferment carbohydrates to produce ethanol *in vitro*, while yeasts also constitute members of the normal intestinal flora. Therefore, in most previous cases, the presence of a small amount of yeast in faecal samples is regarded as an indication that the isolated yeast strain constituted the causative agent of ABS, regardless of whether its alcohol production ability had been confirmed. The present findings confirm the role of *Klebsiella* in the etiology of ABS (Fig. 5), which will help clinicians to achieve precise diagnosis and treatment of ABS in future cases. Our results also indicate that the use of appropriate antibiotics plus a complex probiotic preparation is sufficient for the treatment of intestinal dysbacteriosis-induced bacterial ABS.

Importantly, endogenous alcohol production could affect brain function through the gut–brain axis, primarily by interfering with the actions of gamma-aminobutyric acid and other neurotransmitters.[39,40] In the present study, some neurotransmitters were significantly increased in ABS patients, supporting the hypothesis that gut bacteria induce the release of neuroactive peptides. These findings also suggest that the earliest symptoms of ABS (e.g., mood changes,

which were cefixime with 1.0 mg/L and 4.0 mg/L (line 3), imipenem with 4.0 mg/L and 16.0 mg/L (lines 4), metronidazole with 1.0 mg/L and 4.0 mg/L (line 5), levofloxacin with 1.25 mg/L and 2.5 mg/L (lines 6), and vancomycin with 4.0 mg/L and 32.0 mg/L (line 7). Fermentation of faecal samples from this patient and a normal individual in YPD medium without antibiotic were used as the controls (Lines 1 and 2). Samples were collected at 8, 16, 24 and 48 h, respectively. Data are expressed as the mean ± SD of three biology replicates. **(b)** The detail treatment strategy of ABS patient C. Amino acid and vitamin C injection were first used for 3 days, followed by oral antibiotic 7 days and complex probiotic preparation for 15 days. **(c)** The copy numbers of *Klebsiella* in faecal samples were tested by digital PCR. The copy numbers of *Klebsiella* in faecal samples were significantly decreased accompanying the decreasing of blood alcohol concentration after the antibiotic use. **(d)** Dynamic changes of intestinal microbiota community before and after treatment. The abundance of genus of *Klebsiella* matched well with the treatment and symptoms.

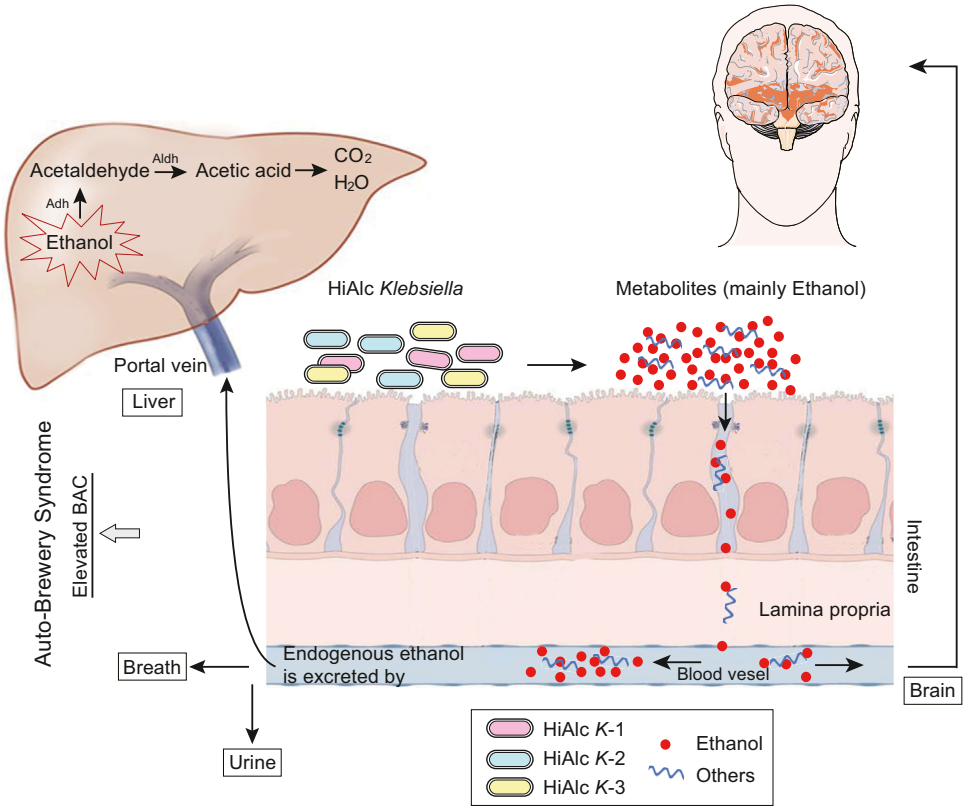

Fig. 5: **The hypothesized mechanism of bacterial ABS.** The intestinal dysbacteriosis resulted proliferation of HiAlc *Klebsiella* after eating alcohol-free high-carbohydrate food and produced a large number of endogenous ethanol, which increases the permeability of intestinal mucosa. Then the metabolites of HiAlc *Klebsiella* and other microbiotas, especially ethanol, enter intestinal blood through lamina propria. The endogenous ethanol diffuses rapidly and uniformly throughout the body water. Some of them were excretion in expires air and urine. The majority of them entered in liver through portal vein and metabolized by Adh and Aldh. Once the amount of endogenous ethanol exceeds the metabolic capacity of the liver, excess ethanol accumulates in the body, resulting in elevated BAC and auto-brewery syndrome. Some metabolites, such as neurotransmitters, could also act on the brain via the gut–brain axis, causing neurological symptoms.

delirium, and brain fog) could be related to overproduction of these neurotransmitters, rather than the medical manifestation of alcohol inebriation.[41]

Study limitations

A key limitation in the present study was only five cases were enrolled. Currently, we continue to recruit more ABS patients and establish a connection with the Auto-Brewery Support Group to support such exploration. A large-scale cohort study is expected to elucidate more evidence in bacterial ABS.

Conclusions

This study reported the characteristics of the gut microbiome and dynamic changes in gut microbiota with bacterial ABS, as well as the effects of therapy. The findings confirm that overgrowth of some intestinal bacteria through producing excessive endogenous alcohol result in the onset of ABS. The use of appropriate antibiotics plus a complex probiotic preparation and a controlled diet may be sufficient for the treatment of bacterial ABS. Furthermore, the endogenous ethanol production is important not only in ABS but also NASH and perhaps many other diseases.

The approach in present study will help clinicians to achieve precise diagnosis of ABS and facilitate future studies of alternative treatments for ABS. While the incidence of ABS is rare, this condition deserves clinical attention because it may result in job loss, relationship difficulties, stigma, incarceration, criminal and other negative social implications.

Contributors

J.Y., R.Y. and J.W. designed and supervised the study. G.X., J.F. and L.G. verified the underlying data, performed statistical analyses. G.X., J.F., R.Z. and B.D. created the study methodology. X.L., S.L., C.L., L.H. and S.D. collected clinical samples clinical data. G.X., J.F., C.Y., Y.F., J.C., H.Z., Z.F., X.C., Z.X., T.F. processed samples and generated experimental data. G.X., J.Y., Y.S. and R.Y. drafted and revised the manuscript. J.Y. and T.Z. obtained funding. All authors have confirmed that they have read and approved the final version of the manuscript.

Data sharing statement

The datasets generated and/or analysed during the current study are available in the https://bigd.big.ac.cn/ under Project ID PRJCA008334/CRA010200.

Declaration of interests

The authors declare that they have no competing interests.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (82130065, 32170201 and 82002191), Beijing Natural Science Foundation (7222014) and FENG Foundation (FFBR 202103) to J.Y.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104560.

References

1 Iwata K. *A review of the literature on Drunken syndromes due to yeasts in the gastrointestinal tract*. Tokyo: University of Tokyo Press; 1972:260–268.
2 Kaji H, Asanuma Y, Ide H, et al. The auto-brewery syndrome–the repeated attacks of alcoholic intoxication due to the overgrowth of Candida (albicans) in the gastrointestinal tract. *Mater Med Pol*. 1976;8(4):429–435.
3 Bouchard Ch. Lectures on autointoxication in disease, or self-poisoning of the individual. *J Am Med Assoc*. 1906;xlvi(4):148.
4 Kaji H, Asanuma Y, Yahara O, et al. Intragastrointestinal alcohol fermentation syndrome: report of two cases and review of the literature. *J Forensic Sci Soc*. 1984;24:461–471.
5 Dahshan A, Donovan K. Auto-brewery syndrome in a child with short gut syndrome: case report and review of the literature. *J Pediatr Gastroenterol Nutr*. 2001;33:214–215.
6 Jansson-Nettelbladt E, Meurling S, Petrini B, Sjolin J. Endogenous ethanol fermentation in a child with short bowel syndrome. *Acta Paediatr*. 2006;95:502–504.
7 Cordell B, McCarthy J. A case study of gut fermentation syndrome (auto-brewery) with Saccharomyces cerevisiae as the causative organism. *Int J Clin Med*. 2013;4:309–312.
8 Cordell B, Kanodia A. Auto-brewery as an emerging syndrome: three representative case studies. *J Clin Med Case Rep*. 2015;2:1–5.
9 Saverimuttu J, Malik F, Arulthasan M, et al. A case of auto-brewery syndrome treated with micafungin. *Cureus*. 2019;11:e5904.
10 Malik F, Wickremesinghe P, Saverimuttu J. Case report and literature review of auto-brewery syndrome: probably an underdiagnosed medical condition. *BMJ Open Gastroenterol*. 2019;6(1):e000325.
11 Cederbaum AI. Alcohol metabolism. *Clin Liver Dis*. 2012;16(4):667–685.
12 Patel S, Behara R, Swanson GR, Forsyth CB, Voigt RM, Keshavarzian A. Alcohol and the intestine. *Biomolecules*. 2015;5(4):2573–2588.
13 Baraona E, Julkunen R, Tannenbaum L, Lieber CS. Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. *Gastroenterology*. 1986;90(1):103–110.
14 Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457:480–484.
15 Lepage P, Hasler R, Spehlmann ME, et al. Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology*. 2011;141:227–236.
16 Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55–60.
17 Natividad JM, Agus A, Planchais J, et al. Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. *Cell Metab*. 2018;28:737–749.e4.
18 Yuan J, Chen C, Cui J, et al. Fatty liver disease caused by high-alcohol-producing Klebsiella pneumoniae. *Cell Metab*. 2019;30:675–688.e7.
19 Bayoumy AB, Mulder CJJ, Mol JJ, Tushuizen ME. Gut fermentation syndrome: a systematic review of case reports. *United European Gastroenterol J*. 2021;9(3):332–342.
20 Dinis-Oliveira RJ. The auto-brewery syndrome: a perfect metabolic "storm" with clinical and forensic implications. *J Clin Med*. 2021;10(20):4637.
21 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30:2114–2120.
22 Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9:357–359.
23 Li D, Liu CM, Luo R, et al. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*. 2015;31:1674–1676.
24 Hyatt D, Chen GL, Locascio PF, et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*. 2010;11:119.
25 Li W, Jaroszewski L, Godzik A. Clustering of highly homologous sequences to reduce the size of large protein databases. *Bioinformatics*. 2001;17:282–283.
26 Jian X, Zhu Y, Ouyang J, et al. Alterations of gut microbiome accelerate multiple myeloma progression by increasing the relative abundances of nitrogen-recycling bacteria. *Microbiome*. 2020;8:74.
27 Jia H, Liu C, Li D, et al. Metabolomic analyses reveals new stage-specific features of the COVID-19. *Eur Respir J*. 2022;59(2):2100284.
28 Simic M, Ajdukovic N, Veselinovic I, et al. Endogenous ethanol production in patients with diabetes mellitus as a medicolegal problem. *Forensic Sci Int*. 2012;216:97–100.
29 Chen H, Wu R, Xing Y, et al. Influence of different inactivation methods on severe acute respiratory syndrome coronavirus 2 RNA copy number. *J Clin Microbiol*. 2020;58(8):e00958-20.
30 Yuan J, Wang B, Sun Z, et al. Analysis of host-inducing proteome changes in bifidobacterium longum NCC2705 grown in vivo. *J Proteome Res*. 2008;7:375–385.
31 Akbaba M. A medicolegal approach to the very rare auto-brewery (endogenous alcohol fermentation) syndrome. *Traffic Inj Prev*. 2020;21(5):295–297.
32 Akhavan BJ, Ostrosky-Zeichner L, Thomas EJ. Drunk without drinking: a case of auto-brewery syndrome. *ACG Case Rep J*. 2019;6:e00208.
33 Kruckenberg KM, DiMartini AF, Rymer JA, et al. Urinary auto-brewery syndrome: a case report. *Ann Intern Med*. 2020;172:702–704.
34 Smędra A, Trzmielak M, Góralska K, Dzikowiec M, Brzeziańska-Lasota E, Berent J. Oral form of auto-brewery syndrome. *J Forensic Leg Med*. 2022;87:102333.
35 Takahashi G, Hoshikawa K, Kan S, et al. Auto-brewery syndrome caused by oral fungi and periodontal disease bacteria. *Acute Med Surg*. 2021;8:e652.
36 Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology*. 2013;57(2):601–609. https://doi.org/10.1002/hep.26093.
37 Mbaye B, Borentain P, Magdy Wasfy R, et al. Endogenous ethanol and triglyceride production by gut Pichia kudriavzevii, Candida albicans and Candida glabrata yeasts in non-alcoholic steatohepatitis. *Cells*. 2022;11(21):3390.
38 Meijnikman AS, Davids M, Herrema H, et al. Microbiome-derived ethanol in nonalcoholic fatty liver disease. *Nat Med*. 2022;28(10):2100–2106.
39 Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*. 2012;13:701–712.
40 Mukherjee S, Vaidyanathan K, Vasudevan DM, et al. Role of plasma amino acids and gaba in alcoholic and non-alcoholic fatty liver disease-a pilot study. *Indian J Clin Biochem*. 2010;25:37–42.
41 Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci U S A*. 2014;111:E4485–E4493.