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Research article

Alterations of mouse gut microbiome in alveolar echinococcosis

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ABSTRACT

Alveolar echinococcosis (AE) may affect the composition of the host's gut microbiota, potentially disrupting the balance between the gut microbiota and metabolites. Metagenomics and untargeted metabolomics were employed to characterize changes in the gut microbiota and metabolites in mouse models infected with *E. multilocularis*. Pearson correlation coefficients were calculated to compare the distribution of microbiota and metabolites, revealing synergistic or mutually exclusive relationships. Functional outputs of the gut microbiota were explored using the CAZy database and six enzymes involved in carbohydrate metabolism were identified with statistically significant differential expression between infected and control groups. The resistome was characterized by identifying antibiotic resistance genes annotated in the Comprehensive Antibiotic Resistance Database from the metagenomes of the groups. Firmicutes are the main carrier of ARGs in the host gut with *tetQ* being most prevalent. Antibiotic efflux, inactivation and target modification were the principal mechanisms of resistance. Comparison and analysis of two sets of antibiotic metabolic pathways allowed the identification of enzyme reactions unique to infected mice. KEGG pathway overview shows phenazine biosynthesis involving phzG to be one of them. In conclusion, infection with AE in mice leads to an overall disruption of gut microbiota and metabolites with the involvement of enzymes related to carbohydrate metabolism. Furthermore, antibiotic-resistance genes may play a role in disease progression, offering potential insights into the relationship between antibiotic use in AE and treatment outcomes.

1. Introduction

Alveolar echinococcosis (AE) is a highly invasive zoonotic disease caused by infection with *E. multilocularis* and has been reported in both developed and developing countries with a hotspot in Western China [1–3]. The widespread distribution of *E. multilocularis* in humans worldwide has raised significant public health concerns. Enhancing our understanding of *E. multilocularis* and exploring measures for the prevention and treatment of AE is of paramount importance.

Dysbiosis of gut microbiota is associated with many health conditions [4] and the parasitic disease, schistosomiasis, has been shown to reduce the richness and diversity of gut microbiota [5]. Analysis of Amazon fish (*Mesonauta festivus*) gut microbiota revealed

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that infection with *Nyctotherus* sp. leads to dysbiosis of the host's intestinal flora while effects of nematodes and flukes are less significant. Furthermore, the host's genetic background was found to have a greater impact on the structure of the gut microbiota than environmental factors [6]. In addition, other parasitic infections also disrupt the balance of the gut microbiota and lead to detectable excretory-secretory products, such as the unusual carbohydrate "talose" found in dog roundworms, a potential biomarker for toxoplasmosis [7].

Parasites and microbes interact in various ways and gut microbiota affect immune modulation triggered by parasites. Parasite-microbe interactions affect gut microbiota, influencing human health through mechanisms such as nutrient metabolism, protection against pathogens and both innate and adaptive immune responses [8,9]. The gut microbiota is essential for maintaining gastrointestinal function and integrity, immune homeostasis and host energy metabolism [10,11]. Studies have found that *E. multilocularis* can cause significant changes in the structure and metabolic capacity of gut microbiota, producing more Treg cell regulators (short-chain fatty acids) and exerting anti-inflammatory effects [12]. Beyond immune modulation, there are other interactions between parasites and microbes. Dalia S [13] reviewed milestone discoveries, such as bacterial infections originating from bacteria within parasites, such as *Salmonella* infections during schistosomiasis. Other bacteria have co-evolved with parasites to cause human diseases, such as *Wolbachia* endosymbionts and filarial nematodes, and the interaction between Gram-negative bacteria and *Schistosoma haematobium* in the pathogenesis of bladder cancer. Some parasitic worms release antimicrobial products which may exacerbate dysbiosis of gut microbiota, affecting the host's susceptibility to other pathogens and overall health [13]. Consequently, antibiotic treatments may indirectly affect parasite infections by altering the gut microbiota, influencing the immune response and the parasites' growth environment. A two-day antibiotic enema treatment has been found to eliminate *B. hominis* and *D. fragilis* and alleviate associated symptoms [14] with positive clinical outcomes, potentially by affecting the gut microbiota and thus eradicating parasites. Therefore, understanding changes in antibiotic-resistance genes within the gut microbiota could provide clues for developing new treatment strategies against these infections.

Altered metabolic status on AE infection affects the gut microenvironment [15]. Changes in the gut microbiota and its metabolites in the feces of mice following infection with *E. multilocularis* were inevtsigated. An analysis of antibiotic resistance genes in both groups was also conducted to investigate the relationship between AE and gut microbiota and guide future clinical treatment.

2. Materials and methods

2.1. Construction of infection models

The current study was conducted in the Key Laboratory of Echinococcosis Research in Qinghai Province, China. Ten female ten-week-old BALB/c mice were reared in a specific pathogen-free (SPF) environment and randomly assigned to two groups ($n = 5$). Each mouse was raised in a clean mouse cage (320 × 215 × 170 mm) with free access to drinking water and commercial mouse feed. One group (EG) was given an intraperitoneal injection of 1000 infectious units of *E. multilocularis* in 200 μL and the second control group (CG) was injected with an equal volume of saline. Three months later, fresh fecal samples were collected daily from each mouse until the total weight reached 5 g. The samples were then quickly frozen in liquid nitrogen and stored at −80 °C. Ultrasound scans of abdominal lesions in EG mice, followed by necropsy were performed to confirm successful establishment of the infection model.

2.2. Metagenomics sequencing

DNA was extracted using a TIANamp Genomic DNA Kit (50preps, DP712-01) and metagenome sequencing was performed for analysis of taxonomy and potential function of the gut microbiome (methods given in online supplementary materials).

2.3. Untargeted metabolomics detection

Untargeted metabolomic analysis was performed on EG and CG fecal samples using Metabolon's Precision Metabolomics liquid chromatography-mass spectrometry (LC-MS/MS) metabolomics platform (methods given in online supplementary materials).

2.4. Correlation analysis

Genera showing significant differences from metagenomics analysis and metabolites showing significant differences from metabolomics analysis were analyzed at the genus level by Pearson correlation coefficients (R value range: 1 to 1). Two experimental variants were negatively correlated when $r < 0$ and positively correlated when $r > 0$. If $r = 0$, no correlation is shown. A heat map was generated to correlate species diversity and metabolites.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 27.0 software (IBM, Chicago, IL, USA) R programming (V 0.3.6.1) and significant differences were shown by *t*-test with $P < 0.05$ considered to demonstrate significant difference and $P < 0.01$ greater significant difference.

3. Results

3.1. Construction of infection models

Ultrasound revealed the presence of typical multilocular cystic structures in the abdominal cavity of EG mice (Fig. 1), confirmed by necropsy, verifying the successful establishment of the AE infection model.

3.2. Alterations in gut microbiota

Metagenomic sequencing gave a mean 11,081.92 million bases per sample for EG and 10,402.47 million bases per sample for CG. In order to examine the distribution of gene counts between the two groups, a Venn diagram was plotted, the two groups share a total of 1,359,129 genes with 188,845 genes unique to EG and 136,442 genes unique to CG (Fig. 2).

Selecting the top 10 microbial taxa with the highest relative abundance present in both groups and labeling the remaining taxa as 'Others', bar charts were generated depicting the relative abundance of species annotations for each sample at different taxonomic levels. Despite the similarity in microbial community composition between the two groups, inter-group differences were observed at genus and species levels (Fig. 3a and b). At the genus level, relative abundances of *Alistipes* (7.34 %), *Clostridium* (4.48 %), *Odoribacter* (1.84 %), *Oscillibacter* (1.61 %) and *Akkermansia* (0.47 %) were higher in EG compared to CG while *Bacteroides* (10.56 %), *Prevotella* (5.46 %), *Lactobacillus* (2.73 %), *Eubacterium* (1.19 %) and *Parabacteroides* (0.92 %) were lower. At the species level, the overall relative abundance of EG (10.67 %) microbiota was lower than CG (17.83 %).

The LAD cladogram revealed that bacteria abundant in EG were mainly distributed in the *Bacteroidales* order, *Erysipelotrichales* order and *Erysipelotrichia* class and in those abundant in CG in the *Lentimicrobiaceae* family and *Cytophagia* class. Others were unclassified (Fig. 3c). Thus, microbiota of both groups primarily resided within the *Firmicutes* phylum and *Bacteroidetes* phylum. To identify bacteria with significant inter-group differences, LDA (Linear Discriminant Analysis) scores (>4) were obtained through LEfSe (Fig. 4). At the genus level, Alistipes had the highest LDA score in EG, followed by *Odoribacter*, *Ruminococcus*, *Lentimicrobium* and *Butyricimonas*. Additionally, at the species level, 9 bacterial species were detected with *Alistipes senegalensis* having the highest LDA score in EG. However, in CG, *Bacteroides* sp. *CAG:927* had the highest LDA score, followed by *Prevotella* sp. *CAG:1031*.

3.3. Alterations in fecal metabolites

To determine whether there was a change in fecal metabolites in mice following AE infection, non-targeted metabolomics analysis was conducted on fecal samples. Principal component analysis (PCA) of differential metabolites showed little variation within groups but significant differences between them (Fig. 5a and b). Z-score analysis based on relative metabolite levels revealed a relatively stable metabolite content in CG while greater dispersion in EG suggested disrupted gut metabolism (Fig. 6a and b).

A total of 609 positive ion mode metabolites and 290 negative ion mode metabolites were identified in the 10 samples. Differential metabolites were screened based on the criteria of VIP >1.0, FC (fold change) > 1.2, or FC < 0.833 and $p < 0.05$. 64 positive ion mode metabolites showed significant differences between the two groups with 50 metabolites upregulated and 14 metabolites downregulated. 35 negative ion mode metabolites exhibited significant differences with 26 upregulated and 9 downregulated. The differentially significant metabolites (top 20 selected) and related information are given in Tables 1 and 2.

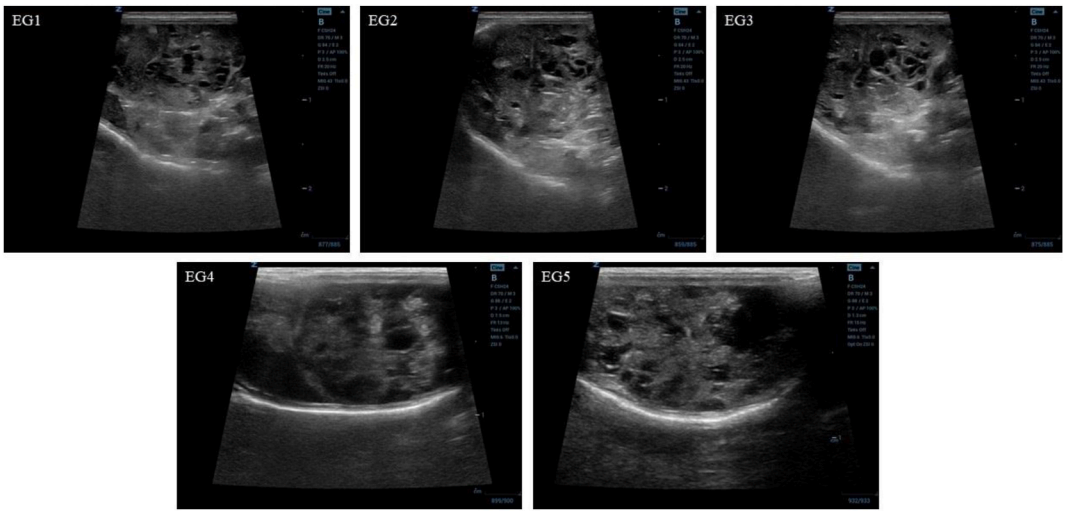


Fig. 1. Examples of abdominal ultrasound images from infected mice. EG1-EG5 present multilocular cystic structures in the abdominal cavity of each infected mouse.

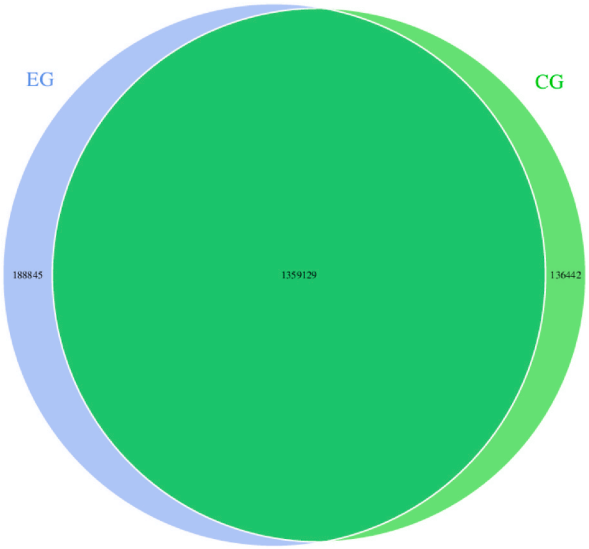


Fig. 2. Venn diagram of the number of genes in the infected and control group. The two groups share 1,359,129 genes, with 188,845 genes unique to the infected group and 136,442 genes unique to the control group. EG: infected group, CG: control group.

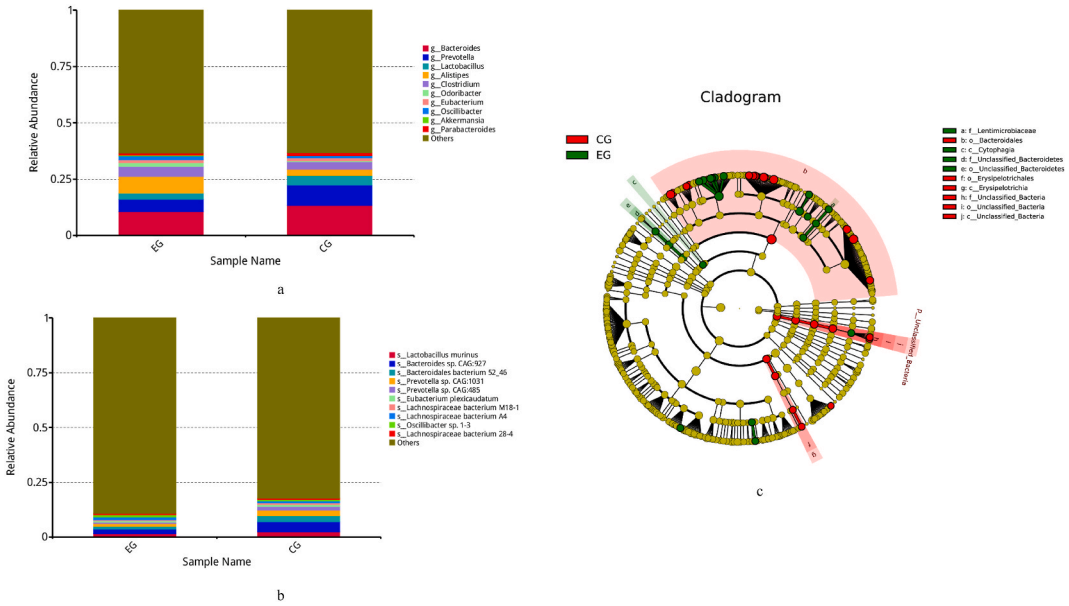


Fig. 3. Relative abundance of microbiota at the genus (a) and species (b) levels. (c) Evolutionary cladogram of differential species. The circles radiating from the inside to the outside represent the taxonomic levels from phylum to species (f, family; o, order; c, class). EG, infected group; CG, control group.

Metabolites with significant fold change differences were represented by a matchstick plot. In summary, both upregulation and downregulation were observed in relation to some aromatic compounds, steroid hormones, amino acids, lipids, vitamins and bile acids (Fig. 7). In positive and negative ion modes, Pleuromutilin and cortodoxone were the most significantly upregulated while pyridoxine O-glucoside and DL-3,4-dihydroxymandelic acid were the most significantly downregulated. Fecal metabolites, such as methyl 3-indolyacetate and phellamurin, originate from food components and may be related to the trends observed in Fig. 7, possibly indicating a dietary influence on metabolites.

3.4. Gut microbiota function outputs

Functional outputs of gut microbiota were explored by CAZy database and six enzymes with statistically significant differential

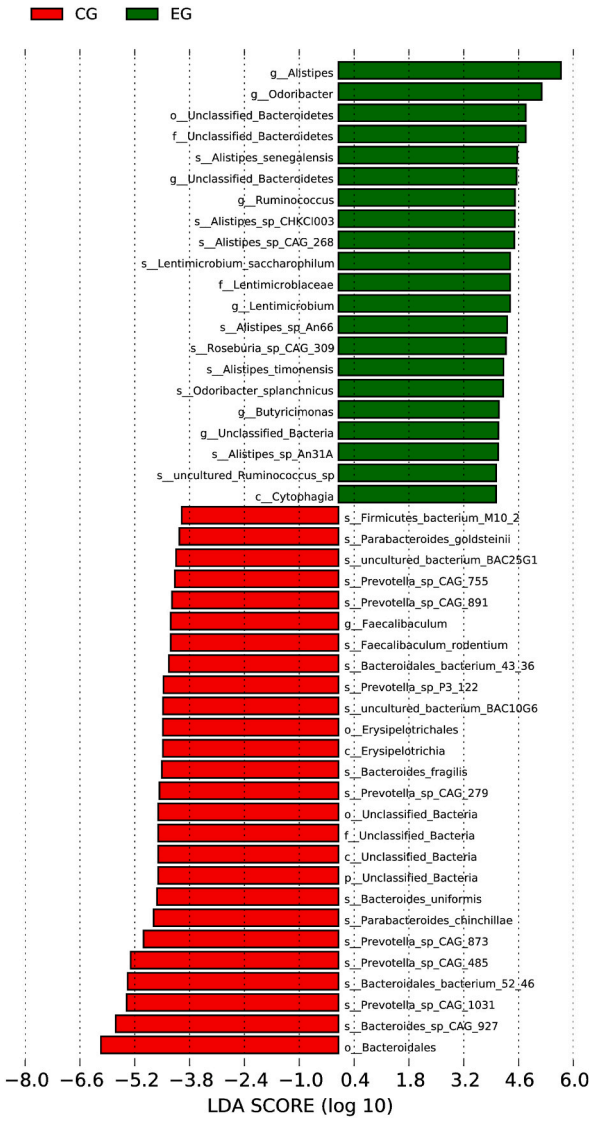
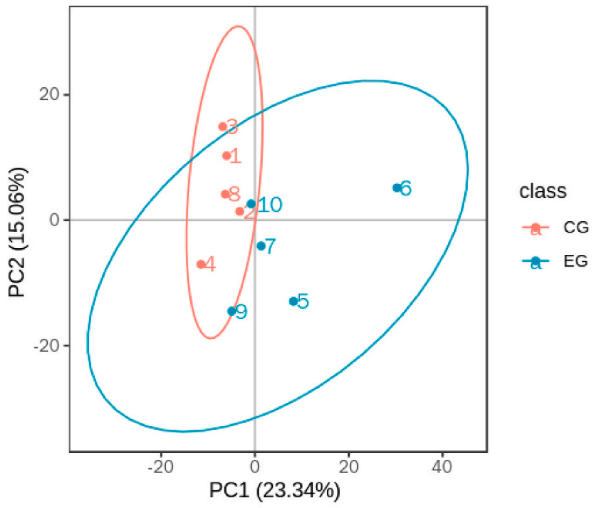


Fig. 4. The histogram of distribution with linear discriminant analysis (LDA) value ≥ 4 (p, phylum; c, class; o, order f, family; g, genus; s, species).

expression were identified between the two groups (Fig. 8). The numbers in the GH (Glycoside Hydrolases) family represent different classes of glycoside hydrolases with individual structural and functional characteristics. According to the CAZy database, the GH18 mainly includes chitinases and lysozymes which act on chitin and peptidoglycan, breaking down these complex polysaccharides. The GH35 primarily consists of β-galactosidases which degrade lactose and β-galactosides. The GH53 includes endo-β-1,4-galactanases which act on β-1,4-galactan. The main members of the GH39 are β-xylosidases which act on β-xylosides. The GH14 is mainly composed of amylases, capable of breaking down starch and glycogen. The GH6 mainly includes cellulases which act on cellulose. Relative abundance of GH18, GH35 and GH39 was higher in EG than in CG while the relative abundance of GH53, GH14 and GH6 was lower (Fig. 8). This indicates that AE leads to significant changes in carbohydrate metabolism with polysaccharides being primarily affected, followed by oligosaccharides.

3.5. Correlation of microbiota and differential metabolites

Pearson correlation coefficients were calculated to compare microbiota and metabolite distribution and indicate synergistic or mutually exclusive relationships (Fig. 9). For example, a significant positive correlation was found between Alistipes and 8-Isoprostaglandin F1α and Lysopc 14:0 ($p < 0.05$) and a negative correlation with an aromatic compound, 5-(3-chloro-4-methylanilino)-1-methyl-1H-pyrazol-3-ol ($p < 0.05$). In addition, Dl-3,4-Dihydroxymandelic Acid was negatively correlated with Arenibacter and Alistipes ($P < 0.05$).



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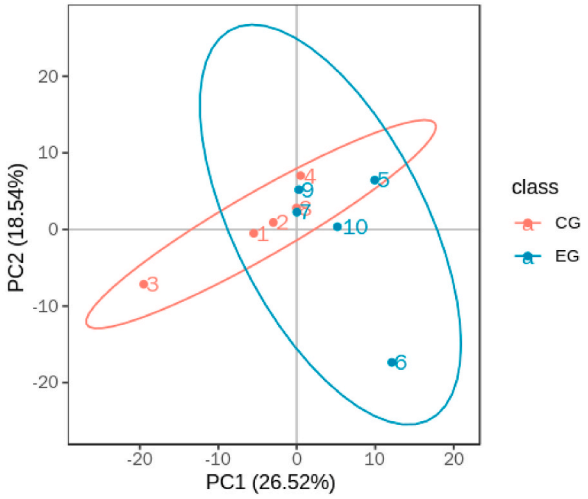


b

Fig. 5. Metabolite PCA. Ellipses indicate the 95 % confidence interval. a) positive ion mode. b) negative ion mode.

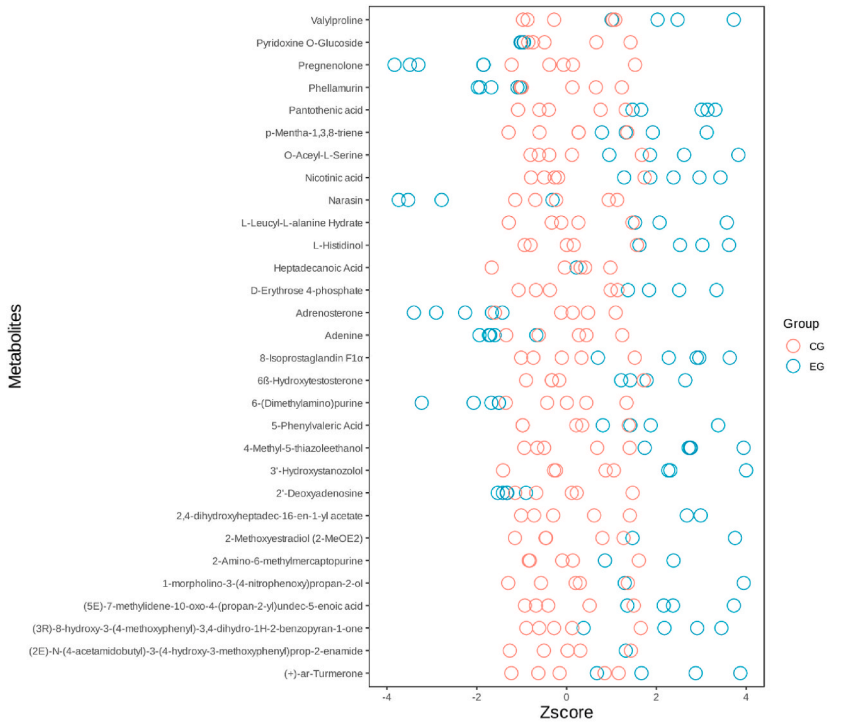
3.6. Gut microbiota antibiotic metabolism

The resistome was characterized by identifying antibiotic resistance genes (ARG) annotated in CARD (Comprehensive Antibiotic Resistance Database) from the metagenomes of each sample. The predominant ARG found was *tetQ* (24.33 %) which was present in samples from both groups and more abundant in EG (Fig. 10a). A circos plot of relationships between antibiotic resistance ontology (ARO) resistance mechanisms and microbiota was constructed. ARG mechanisms in *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were primarily related to antibiotic efflux, inactivation and target modification. The ARG mechanisms of *Firmicutes* (29.80 %) constituted the largest proportion, followed by *Bacteroidetes* (13.04 %) and *Proteobacteria* (14.29 %) (Fig. 10b).

Comparison and analysis of two sets of antibiotic metabolic pathways allowed identification of enzyme reactions disticnbtive to EG in the KEGG pathway overview diagram—phenazine biosynthesis (Fig. 11a), in which phzG is involved (Fig. 11b).

4. Discussion

Parasitic infections may affect the composition of the host's gut microbiota, potentially disrupting the balance between the gut microbiota and the host's immune system [16]. Metagenomics and metabolomics were used to characterize gut microbiota and its metabolites in AE mice. Metagenomic analysis at genus and species levels gave higher resolution and precise functional predictions. Gut microbiota of BALB/c mice changed after *E. multilocularis* infection, indicating bacteria that may be involved in host-parasite



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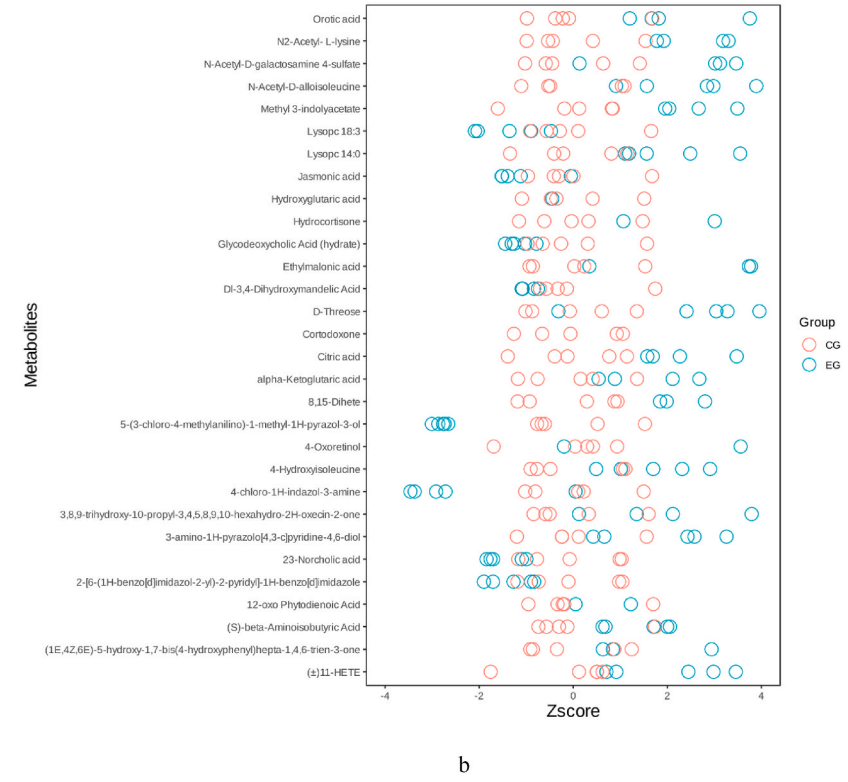

b

Fig. 6. Z-score plot of two groups metabolite. Each circle in the plot represents a sample, and only the top 30 metabolites (ranked by *P*-value in ascending order) with Z-score values (from −4 to 4) are displayed. (a) positive ion mode. (b) negative ion mode.

Table 1
Differential metabolites in positive ion mode for two groups (top 20, ranked by *P*-value in ascending order).

Number	Name	RT (min)	*m/z*	FC	*P*-value	VIP	Up/Down
1	2,4-dihydroxyheptadec-16-en-1-yl acetate	8.029	351.249	2.081	0.001	2.037	up
2	3′-Hydroxystanozolol	5.367	345.253	1.555	0.002	2.005	up
3	1-morpholino-3-(4-nitrophenoxy)propan-2-ol	4.719	283.129	1.211	0.002	2.020	up
4	L-Histidinol	2.517	142.097	1.591	0.002	1.998	up
5	(5E)-7-methylidene-10-oxo-4-(propan-2-yl)undec-5-enoic acid	5.618	253.180	1.527	0.004	1.913	up
6	Pregnenolone	7.868	317.247	0.516	0.004	1.975	down
7	Nicotinic acid	1.871	124.039	1.338	0.004	1.940	up
8	(2E)-N-(4-acetamidobutyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide	5.740	329.147	1.582	0.005	1.950	up
9	Pantothenic acid	5.067	220.117	1.438	0.005	1.934	up
10	4-Methyl-5-thiazoleethanol	1.347	144.048	1.836	0.005	1.992	up
11	Adrenosterone	6.890	301.179	0.655	0.005	1.897	down
12	O-Aceyl-L-Serine	1.612	148.060	1.567	0.006	1.865	up
13	2′-Deoxyadenosine	3.201	252.109	0.232	0.007	1.901	down
14	Valylproline	4.816	215.139	1.873	0.008	1.879	up
15	Phellamurin	3.833	519.179	0.457	0.009	1.832	down
16	6??-Hydroxytestosterone	8.120	305.208	1.649	0.010	1.798	up
17	Adenine	1.440	136.061	0.337	0.010	1.856	down
18	8-Isoprostaglandin F1α	7.288	339.250	1.975	0.010	1.862	up
19	(+)-*ar*-Turmerone	5.619	217.159	1.506	0.011	1.787	up
20	2-Amino-6-methylmercaptopurine	6.747	182.049	2.015	0.013	1.843	up

Table 2
Differential metabolites in negative ion mode for two groups (top 20, ranked by *P*-value in ascending order).

Number	Name	RT (min)	*m/z*	FC	*P*-value	VIP	Up/Down
1	5-(3-chloro-4-methylanilino)-1-methyl-1H-pyrazol-3-ol	4.901	236.060	0.373	<0.001	2.206	down
2	Cortodoxone	6.351	345.207	6.866	0.001	2.221	up
3	8,15-Dihete	8.170	335.223	1.983	0.005	1.825	up
4	Methyl 3-indolyacetate	5.542	188.072	1.675	0.006	1.799	up
5	Dl-3,4-Dihydroxymandelic Acid	5.009	183.030	0.183	0.006	1.798	down
6	23-Norcholic acid	7.047	393.265	0.322	0.009	1.707	down
7	Orotic acid	1.494	155.010	2.376	0.009	1.690	up
8	N2-Acetyl- L-lysine	1.408	187.109	1.978	0.011	1.738	up
9	Glycodeoxycholic Acid (hydrate)	7.625	448.306	0.307	0.012	1.660	down
10	N-Acetyl-D-alloisoleucine	5.712	172.098	1.926	0.013	1.681	up
11	Hydrocortisone	6.580	361.202	3.178	0.013	1.741	up
12	Ethylmalonic acid	5.057	131.035	2.265	0.015	1.693	up
13	Lysopc 14:0	5.832	466.292	1.402	0.019	1.605	up
14	Hydroxyglutaric acid	2.011	147.030	3.472	0.021	1.635	up
15	12-oxo Phytodienoic Acid	8.000	309.207	4.299	0.021	1.639	up
16	(1E,4Z,6E)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-1,4,6-trien-3-one	4.929	261.088	1.663	0.025	1.579	up
17	2-[6-(1H-benzo[*d*]imidazole-2-yl)-2-pyridyl]-1H-benzo[*d*]imidazole	5.929	311.117	0.515	0.025	1.541	down
18	4-chloro-1H-indazol-3-amine	1.426	166.018	0.350	0.026	1.658	down
19	D-Threose	1.353	119.035	1.367	0.027	1.583	up
20	3,8,9-trihydroxy-10-propyl-3,4,5,8,9,10-hexahydro-2H-oxecin-2-one	5.644	243.123	1.480	0.027	1.524	up

interactions. The *Bacteroidetes* phylum aids in the host digestion of complex carbohydrates, bile acid biotransformation, vitamin synthesis and immune system development [17]. However, *E. multilocularis* infection altered *Bacteroidetes* genera in the mouse model, increasing the abundance of *Alistipes* in EG compared with CG. *Alistipes* produces anti-inflammatory metabolites to promote the differentiation of anti-inflammatory Treg/Tr1 cells in the mouse gut [18] and disrupts the gut barrier by increasing trimethylamine N-oxide and reducing the production of short-chain fatty acids [19,20]. A positive correlation was found between *Alistipes* and 8-Isoprostaglandin F1α, thermacetogenium and 3′-Hydroxystanozolol during the current work. Additionally, *Alistipes* has previously been found to increase in mice infected with Schistosoma, perhaps related to host gut inflammation, immune and stress responses [5], a finding which is consistent with those of the current work. The abundance of Alistipes in fecal matter has also been associated with gut dysbiosis during previous studies [21–23].

Bacteroides and *Prevotella* were found to be the dominant genera in healthy mice. Similar to *Bacteroidales* [24–26], *Prevotella* also maintains gut microbiota balance and promotes health. For example, *Prevotella* regulates the host's immune response and influences the gut immune environment, reducing inflammation and maintaining immune balance [27,28]. *Prevotella* also produces short-chain fatty acids by fermenting dietary fibers and carbohydrates and these are beneficial for gut health and host metabolism [29]. This is consistent with the functional output results of the gut microbiota in this study: AE led to significant changes in gut carbohydrate metabolism. However, *Prevotella* has different functions compared with *Bacteroidales* and is associated with high-fiber, plant-based diets. *Prevotella* breaks down complex carbohydrates, utilizing plant-based carbohydrates and protein degradation products [30,31]. This may account for changes in the metabolites, methyl 3-indolyacetate and phellamurin, observed in the current study. Furthermore,

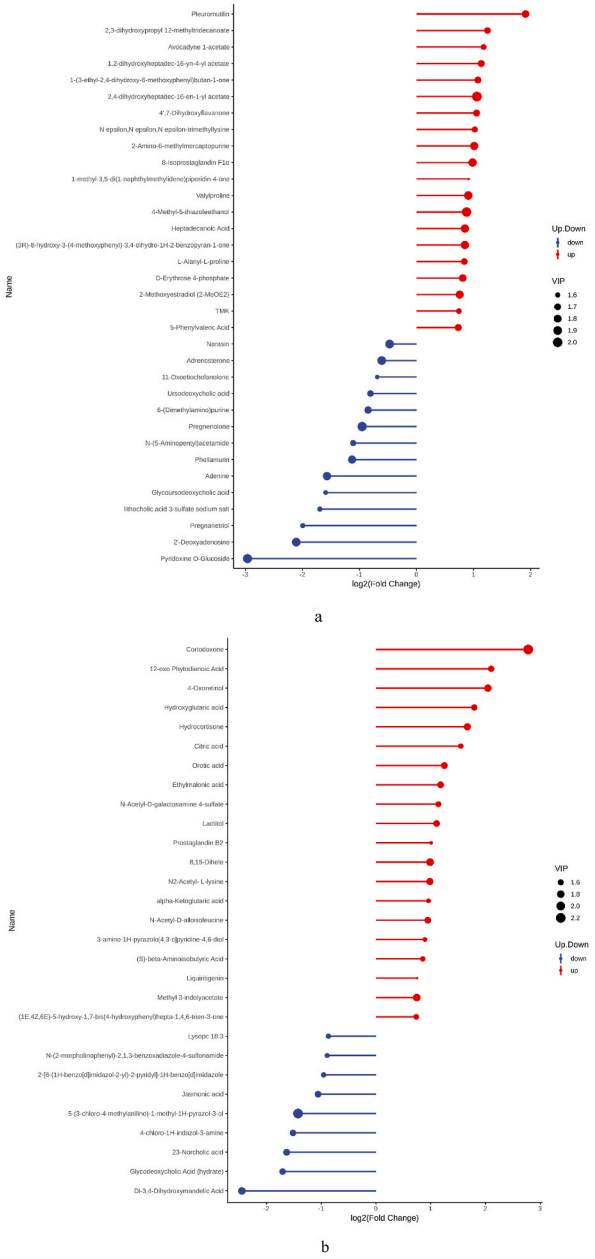

Fig. 7. Metabolite matchstick plot showing differential expression. (a) positive ion mode. (b) negative ion mode.

a negative correlation between levels of inflammatory factors and *Prevotella* in the gut has been found [32]. This suggests that the reason *Prevotella* no longer remains the dominant genus after mice are infected might be due to the high levels of inflammatory factors in the gut.

Gut bacteria may have a significant impact on host metabolism. In current study, disruption of aromatic compounds, steroid hormones, amino acids, lipids, vitamins and bile acids were osberved. Steroid hormones and prostaglandins are involved in the stress response [33,34] which may be triggered by parasitic infection. Therefore, the upregulation of the metabolite cortodoxone may be related to the host's immune response, inflammation, stress and stress-related factors. 43 % of the 137 metabolites in the metabolomics database were involved in amino acid metabolism [35] and amino acids, such as glycine, leucine, serine, cysteine and threonine, are bioactive molecules involved in signaling pathways and metabolic regulation [36]. Therefore, the increase in amino acid metabolites may be associated with the activation of the host's gut immune and signaling responses during AE. Some vitamins, such as biotin, a B-vitamin with roles in gene expression, cell signaling and chromatin structure, were among the metabolites upregulated in the EG. Biotin-dependent signaling pathways regulate the expression of genes involved in apoptosis and cell survival [37]. Gut microbes are known to affect the absorption, transport and utilization of lipids [38,39] and levels of hemolytic phospholipids and unsaturated fatty

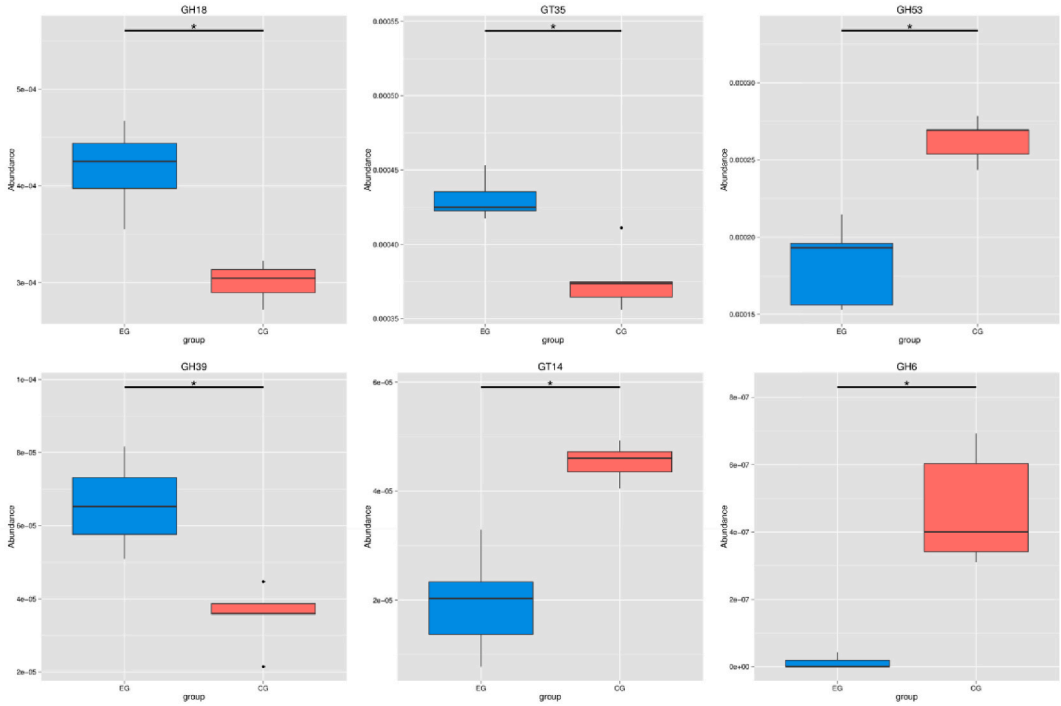

Fig. 8. Functional outputs of gut microbiota were explored by CAZy database. The numbers in the GH (glycoside hydrolases) family represent different types of glycoside hydrolases. GH18 primarily includes chitinases and lysozymes. GH35 primarily consists of β-galactosidases. GH53 primarily includes endo-β-1,4-galactanases. GH39 primarily has β-xylosidases. GH14 primarily consists of amylases, while GH6 primarily includes cellulases. Compared to CG, EG has a higher relative abundance of GH18, GH35, and GH39, and a lower abundance of GH53, GH14, and GH6. This suggests different metabolic outputs between the two groups.★ denotes statistical significance, i.e., $P < 0.05$.

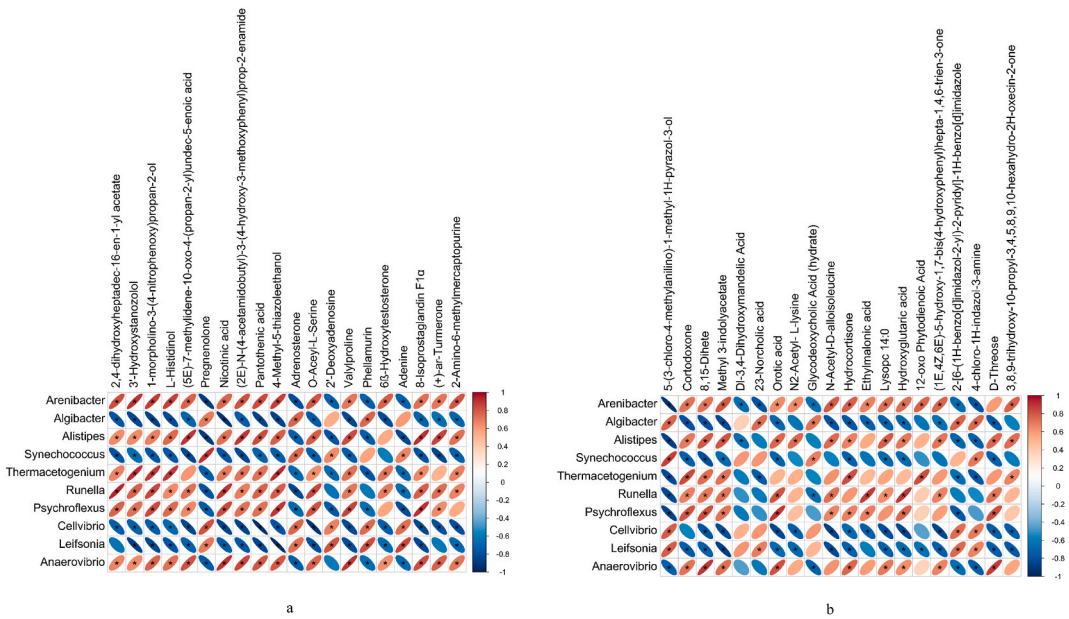

Fig. 9. The correlation between differential species and differential metabolites. Red indicates positive correlation, blue indicates negative correlation, and * denotes statistical significance, i.e., $P < 0.05$. (a) positive ion mode. (b) negative ion mode.

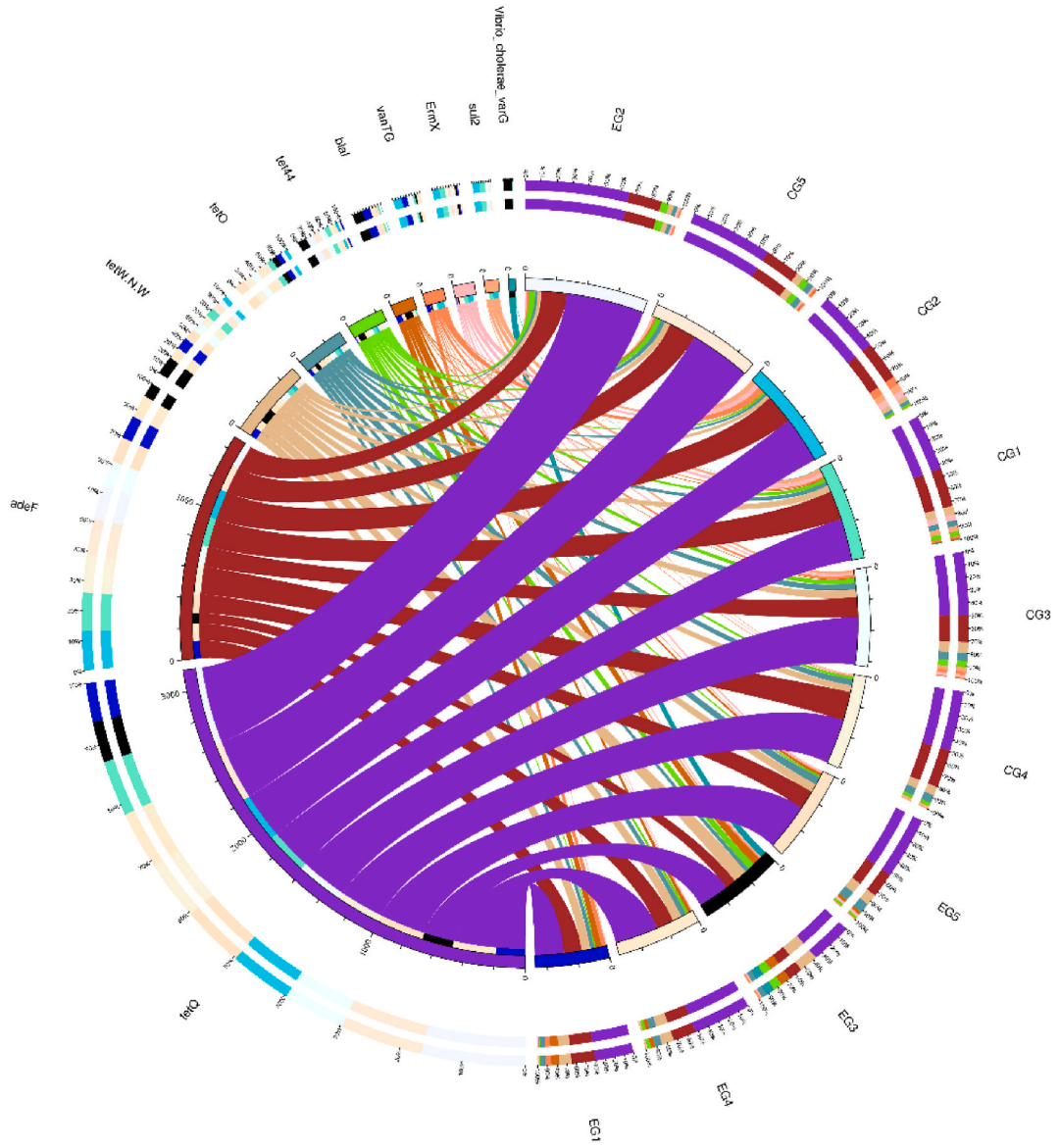

Fig. 10. a Resistance genes overview circos diagram, sample information on the right and ARO information on the left.
b Resistance mechanisms and species overview circos diagram, information on phylum level species is shown on the right, and information on resistance mechanisms is shown on the left.

acids increased in the EG, perhaps due to gut inflammation. Gut dysbiosis was found to cause intestinal inflammation and disruption of intestinal barrier function, leading to bacterial translocation and reducing the conversion of primary to secondary bile acids in the gut [40,41]. Bile acid metabolism was upregulated in the EG mice of the current study, consistent with previous research findings.

Some differences and distinctive characteristics emerged from the comparison of antibiotic resistance genes in EG and CG. *Firmicutes* were the main carrier of ARGs in the host gut with *tetQ* type being most prevalent and *adeF* the second most abundant. Antibiotic efflux, inactivation and target modification were the principal mechanisms of resistance. *tetQ* and *adeF* are common bacterial resistance genes and encode efflux pumps for tetracycline and aminoglycoside, respectively [42,43], allowing bacteria to expel these drugs from the cell. Additionally, phzG catalyzes an oxidation/aromatization reaction in pyrazine metabolism and was involved in the antibiotic metabolism pathway of EG Ref. [44]. phzG is known to decarboxylate and activate antibacterial compound precursors [45], Therefore, the phzG antibiotic metabolic pathway involving AE may be related to the synthesis of bioactive compounds. We believe that understanding the function of phzG and its role in parasitic disease gut microbiota could indicate new therapeutic strategies against parasitic infections. Interfering with antibiotic metabolism-related genes or microbial communities in the parasitic disease gut microbiota may potentially impact the progression and treatment outcomes of parasitic infections.

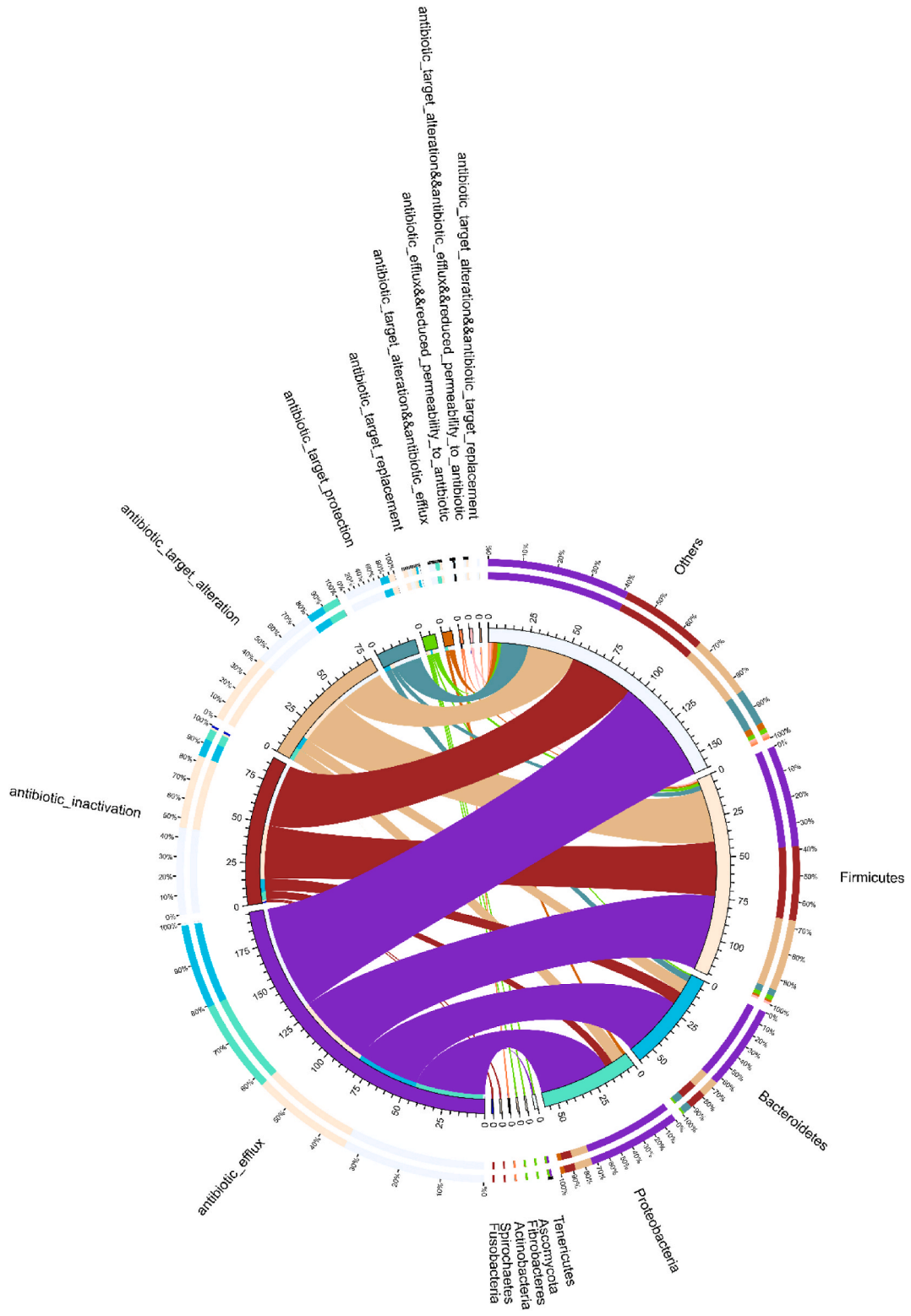

Fig. 10. (*continued*).

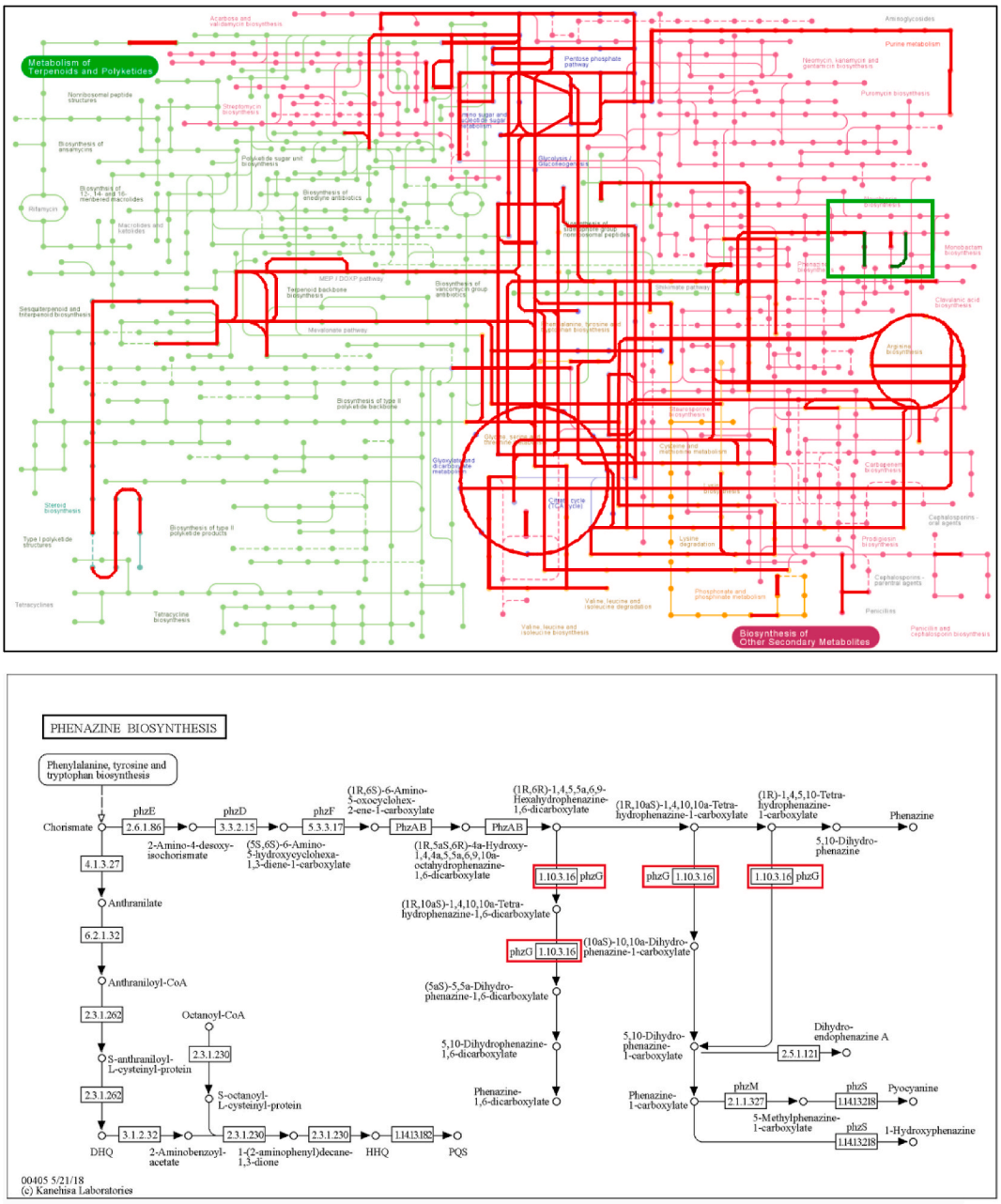

Fig. 11. a iPath diagram of antibiotic biosynthesis pathways, The thick red line indicates enzyme reactions common to both the infected and control groups, while the thick green line indicates enzyme reactions specific to the infected group (marked with a green box). The enzyme corresponding to these reactions is phzG. phzG is involved in phenazine biosynthesis, with the detailed synthesis process shown in Fig. 11b
b Phenazine biosynthesis. The red border has been used to mark the location of phzG. Information extracted from https://www.genome.jp/pathway/map00405.

We acknowledge some limitations to the current study. Firstly, AE may indirectly influence gut microbiota via the host's immune response, especially for bacteria associated with the host gut epithelium. Hence, microbiota in different regions of the intestinal lumen and attached epithelium should be scrutinized to determine the full impact of *E. multilocularis* infection. Secondly, other omics data, such as proteomics, may complement and guide the interpretation and linkage of metagenomic and metabolomic data. Therefore, in subsequent studies, more advanced technologies and methods, such as single-cell sequencing, multi-omics integrative analysis and high-throughput screening, should be utilized in mechanistic investigations. Finally, although the host's genetic background is thought to have a greater impact on the structure of the gut microbiota than environmental factors, factors such as mouse strain, diet and facility conditions still influence the baseline microbial ecology and response to interventions. Therefore, in designing and conducting

research, these factors should be fully considered to more accurately analyze the dynamic changes in microbial communities and their effects on the host, giving new perspectives and strategies for AE treatment and health management. In conclusion, AE induces significant changes in the host gut microbiota, affecting functions and related metabolites, indicating an imbalance in the gut ecosystem. Enzymes related to carbohydrate metabolism are involved and antibiotic resistance genes may play a role in disease progression, providing potential insights into the relationship between antibiotic use and treatment outcomes in AE.

Ethics statement

This study strictly follows the policies and laws of the National Institutes of Health (NIH) Office of Laboratory Animal Welfare and complies with the ARRIVE guidelines. The study has been approved by the Ethics Committee of Qinghai University Affiliated Hospital, China (Approval No. P-SL-2019042).

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Availability of data and materials

The data that support the findings of this study are openly available in Mendeley Data, https://doi.org/10.17632/mj7j53c9g3.1.

CRediT authorship contribution statement

Ziyan Cui: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Fei Du:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Wenhao Yu:** Supervision, Formal analysis, Data curation. **Zhixin Wang:** Supervision, Methodology. **Fanyu Kong:** Methodology, Data curation. **Zhi Xie:** Validation. **Qian Zhao:** Validation. **Hanxi Zhang:** Validation. **Haijiu Wang:** Validation. **Haining Fan:** Validation. **Li Ren:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

LiRen reports financial support was provided by The Qinghai Provincial Department of Science and Technology Agency, China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32860.

References

[1] H. Wen, L. Vuitton, T. Tuxun, J. Li, D.A. Vuitton, W. Zhang, D.P. McManus, Echinococcosis: Advances in the 21st Century, Clin. Microbiol. Rev. 32 (2019), https://doi.org/10.1128/CMR.00075-18.

[2] A. Casulli, Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis, Lancet Global Health 8 (2020) e470–e471, https://doi.org/10.1016/S2214-109X(20)30066-8.

[3] B. Dezsényi, Z. Dubóczki, T. Strausz, E. Csulak, V. Czoma, Z. Káposztás, M. Fehérvári, Á. Somorácz, A. Csilek, A. Oláh, K. Almási, A. Patonai, D. Görög, Z. Széll, Z. Tolnai, T. Sréter, J. Danka, H. Auer, B. Grüner, T.F.E. Barth, A. Casulli, Emerging human alveolar echinococcosis in Hungary (2003–2018): a retrospective case series analysis from a multi-centre study, BMC Infect. Dis. 21 (2021) 168, https://doi.org/10.1186/s12879-021-05859-5.

[4] J.C. Clemente, L.K. Ursell, L.W. Parfrey, R. Knight, The impact of the gut microbiota on human health: an integrative View, Cell 148 (2012) 1258–1270, https://doi.org/10.1016/j.cell.2012.01.035.

[5] Y. Hu, J. Chen, Y. Xu, H. Zhou, P. Huang, Y. Ma, M. Gao, S. Cheng, H. Zhou, Z. Lv, Alterations of gut microbiome and metabolite Profiling in mice infected by schistosoma japonicum, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.569727.

[6] N. Leroux, F.-E. Sylvain, A. Holland, A. Luis Val, N. Derome, Gut microbiota of an Amazonian fish in a heterogeneous riverscape: integrating genotype, environment, and parasitic infections, Microbiol. Spectr. 11 (2023), https://doi.org/10.1128/spectrum.02755-22.

[7] P. Wangchuk, O. Lavers, D.S. Wishart, A. Loukas, Excretory/secretory metabolome of the zoonotic roundworm parasite Toxocara canis, Biomolecules 10 (2020) 1157, https://doi.org/10.3390/biom10081157.

[8] L.V. Hooper, D.R. Littman, A.J. Macpherson, Interactions between the microbiota and the immune system, Science 336 (2012) 1268–1273, https://doi.org/10.1126/science.1223490, 1979.

[9] E.M. Brown, M. Sadarangani, B.B. Finlay, The role of the immune system in governing host-microbe interactions in the intestine, Nat. Immunol. 14 (2013) 660–667, https://doi.org/10.1038/ni.2611.

[10] K.M. Maslowski, C.R. Mackay, Diet, gut microbiota and immune responses, Nat. Immunol. 12 (2011) 5–9, https://doi.org/10.1038/ni0111-5.

[11] N. Kamada, S.-U. Seo, G.Y. Chen, G. Núñez, Role of the gut microbiota in immunity and inflammatory disease, Nat. Rev. Immunol. 13 (2013) 321–335, https://doi.org/10.1038/nri3430.

[12] Y. Wang, A. Guo, Y. Zou, W. Mu, S. Zhang, Z. Shi, Z. Liu, X. Cai, X.-Q. Zhu, S. Wang, Interaction between tissue-dwelling helminth and the gut microbiota drives mucosal immunoregulation, NPJ Biofilms Microbiomes 9 (2023) 43, https://doi.org/10.1038/s41522-023-00410-7.

[13] D.S. Ashour, A.A. Othman, Parasite–bacteria interrelationship, Parasitol. Res. 119 (2020) 3145–3164, https://doi.org/10.1007/s00436-020-06804-2.

[14] N. Roshan, A. Clancy, A.W. Gunaratne, A. LeBusque, D. Pilarinos, T.J. Borody, Two-day enema antibiotic therapy for parasite eradication and resolution of symptoms, World J. Gastroenterol. 26 (2020) 3792–3799, https://doi.org/10.3748/wjg.v26.i26.3792.

[15] M.A. Santa, S. Pastran, C. Klein, K. Ruckstuhl, A. Massolo, Evaluation of an automated magnetic bead-based DNA extraction and real-time PCR in fecal samples as a pre-screening test for detection of Echinococcus multilocularis and Echinococcus canadensis in coyotes, Parasitol. Res. 118 (2019) 119–125, https://doi.org/10.1007/s00436-018-6125-y.

[16] V. Guernier, B. Brennan, L. Yakob, G. Milinovich, A.C.A. Clements, R.J. Soares Magalhaes, Gut microbiota disturbance during helminth infection: can it affect cognition and behaviour of children? BMC Infect. Dis. 17 (2017) 58, https://doi.org/10.1186/s12879-016-2146-2.

[17] Q. Emu, H. Guan, J. Zhu, L. Zhang, J. Fan, Y. Ji, Y. Lin, C. Li, X. Dan, Y. Aguo, X. Wei, M. Zhang, B. Zhang, C. Yang, B. Li, C. Xiong, Grazing and Supplementation of dietary Yeast Probiotics shape the gut microbiota and improve the immunity of black fattening goats (Capra hircus), Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.666837.

[18] R. Dziarski, S.Y. Park, D.R. Kashyap, S.E. Dowd, D. Gupta, Pglyrp-regulated gut microflora Prevotella falsenii, Parabacteroides distasonis and Bacteroides eggerthii enhance and Alistipes finegoldii attenuates colitis in mice, PLoS One 11 (2016) e0146162, https://doi.org/10.1371/journal.pone.0146162.

[19] J. Wang, Y. Wang, X. Zhang, J. Liu, Q. Zhang, Y. Zhao, J. Peng, Q. Feng, J. Dai, S. Sun, Y. Zhao, L. Zhao, Y. Zhang, Y. Hu, M. Zhang, Gut microbial dysbiosis is associated with altered hepatic functions and serum metabolites in chronic hepatitis B patients, Front. Microbiol. 8 (2017), https://doi.org/10.3389/fmicb.2017.02222.

[20] C. Chiu, M. Cheng, M. Chiang, Y. Kuo, M. Tsai, C. Chiu, G. Lin, Gut microbial-derived butyrate is inversely associated with IgE responses to allergens in childhood asthma, Pediatr. Allergy Immunol. 30 (2019) 689–697, https://doi.org/10.1111/pai.13096.

[21] Y. Kang, X. Kuang, H. Yan, P. Ren, X. Yang, H. Liu, Q. Liu, H. Yang, X. Kang, X. Shen, M. Tong, L. Li, X. Wang, L. Guo, J. Ma, F. Zhang, W. Fan, A novel synbiotic alleviates autoimmune hepatitis by modulating the gut microbiota-liver Axis and inhibiting the hepatic TLR4/NF-κB/NLRP3 signaling pathway, mSystems 8 (2023), https://doi.org/10.1128/msystems.01127-22.

[22] H. Tokuno, T. Itoga, J. Kasuga, K. Okuma, K. Hasuko, H. Masuyama, Y. Benno, Method for estimating disease risk from microbiome data using structural equation modeling, Front. Microbiol. 14 (2023), https://doi.org/10.3389/fmicb.2023.1035002.

[23] Z. Sun, X. Pan, X. Li, L. Jiang, P. Hu, Y. Wang, Y. Ye, P. Wu, B. Zhao, J. Xu, M. Kong, Y. Pu, M. Zhao, J. Hu, J. Wang, G. Chen, C. Yuan, Y. Yu, X. Gao, F. Zhao, A. Pan, Y. Zheng, The gut microbiome dynamically associates with host glucose metabolism throughout pregnancy: longitudinal findings from a matched case-control study of gestational diabetes mellitus, Adv. Sci. 10 (2023), https://doi.org/10.1002/advs.202205289.

[24] L.V. Hooper, T.S. Stappenbeck, C.V. Hong, J.I. Gordon, Angiogenins: a new class of microbicidal proteins involved in innate immunity, Nat. Immunol. 4 (2003) 269–273, https://doi.org/10.1038/ni888.

[25] N.L. Zitomersky, B.J. Atkinson, S.W. Franklin, P.D. Mitchell, S.B. Snapper, L.E. Comstock, A. Bousvaros, Characterization of adherent Bacteroidales from intestinal biopsies of children and young adults with inflammatory bowel disease, PLoS One 8 (2013) e63686, https://doi.org/10.1371/journal.pone.0063686.

[26] S.K. Mazmanian, C.H. Liu, A.O. Tzianabos, D.L. Kasper, An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system, Cell 122 (2005) 107–118, https://doi.org/10.1016/j.cell.2005.05.007.

[27] J.M. Larsen, The immune response to *Prevotella* bacteria in chronic inflammatory disease, Immunology 151 (2017) 363–374, https://doi.org/10.1111/imm.12760.

[28] J.U. Scher, S.B. Abramson, The microbiome and rheumatoid arthritis, Nat. Rev. Rheumatol. 7 (2011) 569–578, https://doi.org/10.1038/nrrheum.2011.121.

[29] A. Trautmann, L. Schleicher, S. Deusch, J. Gätgens, J. Steuber, J. Seifert, Short-chain fatty acids modulate metabolic pathways and membrane lipids in Prevotella bryantii B14, Proteomes 8 (2020) 28, https://doi.org/10.3390/proteomes8040028.

[30] F. De Filippis, N. Pellegrini, L. Vannini, I.B. Jeffery, A. La Storia, L. Laghi, D.I. Serrazanetti, R. Di Cagno, I. Ferrocino, C. Lazzi, S. Turroni, L. Cocolin, P. Brigidi, E. Neviani, M. Gobbetti, P.W. O'Toole, D. Ercolini, High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome, Gut 65 (2016) 1812–1821, https://doi.org/10.1136/gutjnl-2015-309957.

[31] G.D. Wu, J. Chen, C. Hoffmann, K. Bittinger, Y.-Y. Chen, S.A. Keilbaugh, M. Bewtra, D. Knights, W.A. Walters, R. Knight, R. Sinha, E. Gilroy, K. Gupta, R. Baldassano, L. Nessel, H. Li, F.D. Bushman, J.D. Lewis, Linking long-term dietary patterns with gut microbial enterotypes, Science 334 (2011) 105–108, https://doi.org/10.1126/science.1208344, 1979.

[32] E. Elinav, T. Strowig, A.L. Kau, J. Henao-Mejia, C.A. Thaiss, C.J. Booth, D.R. Peaper, J. Bertin, S.C. Eisenbarth, J.I. Gordon, R.A. Flavell, NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis, Cell 145 (2011) 745–757, https://doi.org/10.1016/j.cell.2011.04.022.

[33] S.L. Lightman, M.T. Birnie, B.L. Conway-Campbell, Dynamics of ACTH and cortisol secretion and implications for disease, Endocr. Rev. 41 (2020), https://doi.org/10.1210/endrev/bnaa002.

[34] E.M. Brady, D.R. Webb, D.H. Morris, K. Khunti, D.S.C. Talbot, N. Sattar, M.J. Davies, Investigating endothelial activation and oxidative stress in relation to glycaemic control in a multiethnic population, Exp. Diabetes Res. 2012 (2012) 1–8, https://doi.org/10.1155/2012/386041.

[35] G. Zaccherini, F. Aguilar, P. Caraceni, J. Clària, J.J. Lozano, F. Fenaille, F. Castelli, C. Junot, A. Curto, C. Formentin, E. Weiss, M. Bernardi, R. Jalan, P. Angeli, R. Moreau, V. Arroyo, Assessing the role of amino acids in systemic inflammation and organ failure in patients with ACLF, J. Hepatol. 74 (2021) 1117–1131, https://doi.org/10.1016/j.jhep.2020.11.035.

[36] J. Sankarasubramanian, R. Ahmad, N. Avuthu, A.B. Singh, C. Guda, Gut microbiota and metabolic specificity in ulcerative colitis and crohn's disease, Front. Med. 7 (2020), https://doi.org/10.3389/fmed.2020.606298.

[37] J. Zempleni, UPTAKE, localization, and NONCARBOXYLASE roles of biotin, Annu. Rev. Nutr. 25 (2005) 175–196, https://doi.org/10.1146/annurev.nutr.25.121304.131724.

[38] F. Bäckhed, H. Ding, T. Wang, L.V. Hooper, G.Y. Koh, A. Nagy, C.F. Semenkovich, J.I. Gordon, The gut microbiota as an environmental factor that regulates fat storage, Proc. Natl. Acad. Sci. USA 101 (2004) 15718–15723, https://doi.org/10.1073/pnas.0407076101.

[39] J. Boursier, O. Mueller, M. Barret, M. Machado, L. Fizanne, F. Araujo-Perez, C.D. Guy, P.C. Seed, J.F. Rawls, L.A. David, G. Hunault, F. Oberti, P. Calès, A. M. Diehl, The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota, Hepatology 63 (2016) 764–775, https://doi.org/10.1002/hep.28356.

[40] P. Chen, P. Stärkel, J.R. Turner, S.B. Ho, B. Schnabl, Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice, Hepatology 61 (2015) 883–894, https://doi.org/10.1002/hep.27489.

[41] M. Úbeda, M. Lario, L. Muñoz, M.-J. Borrero, M. Rodríguez-Serrano, A.-M. Sánchez-Díaz, R. del Campo, L. Lledó, Ó. Pastor, L. García-Bermejo, D. Díaz, M. Álvarez-Mon, A. Albillos, Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats, J. Hepatol. 64 (2016) 1049–1057, https://doi.org/10.1016/j.jhep.2015.12.010.

[42] I. Chopra, M. Roberts, Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance, Microbiol. Mol. Biol. Rev. 65 (2001) 232–260, https://doi.org/10.1128/MMBR.65.2.232-260.2001.

[43] A. Chukamnerd, K. Singkhamanan, V. Chongsuvivatwong, P. Palittapongarnpim, Y. Doi, R. Pomwised, C. Sakunrang, K. Jeenkeawpiam, M. Yingkajorn, S. Chusri, K. Surachat, Whole-genome analysis of carbapenem-resistant Acinetobacter baumannii from clinical isolates in Southern Thailand, Comput. Struct. Biotechnol. J. 20 (2022) 545–558, https://doi.org/10.1016/j.csbj.2021.12.038.

[44] J.F. Parsons, F. Song, L. Parsons, K. Calabrese, E. Eisenstein, J.E. Ladner, Structure and function of the phenazine biosynthesis protein PhzF from *Pseudomonas fluorescens* 2-79, Biochemistry 43 (2004) 12427–12435, https://doi.org/10.1021/bi049059z.

[45] S. Guo, Y. Wang, M. Bilal, H. Hu, W. Wang, X. Zhang, Microbial synthesis of antibacterial phenazine-1,6-dicarboxylic acid and the role of PhzG in *Pseudomonas chlororaphis* GP72AN, J. Agric. Food Chem. 68 (2020) 2373–2380, https://doi.org/10.1021/acs.jafc.9b07657.