

Host-associated gut microbiomes and resistomes in wild birds and sympatric Himalayan marmots from the Qinghai–Tibet Plateau

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Abstract

Background

Wild birds are being increasingly recognized as potential reservoirs and disseminators of antibiotic resistance genes (ARGs); however, the microbiome and resistome profiles of plateau-dwelling wild birds remain insufficiently characterized.

Results

In this study, shotgun metagenomic sequencing was used to investigate the gut microbiomes and resistomes of six wild bird species from the Qinghai–Tibet Plateau, with sympatric Himalayan marmots (*Marmota himalayana*) included for host-comparative analysis. The gut microbiota of the bird species was dominated by Pseudomonadota, Actinomycetota, Bacillota, and Chlamydiota; however, the microbial composition varied markedly among the bird groups. Functional annotation revealed that metabolism, genetic information processing, and environmental information processing were the predominant KEGG categories. CARD annotation revealed 130 antibiotic resistance ontology categories in bird gut microbiomes, including genes associated with multidrug resistance, β -lactams, quinolones, tetracyclines, polymyxins, and glycopeptide resistance. Microbial source analysis revealed that ARGs were associated mainly with Pseudomonadota, Bacillota, and Actinomycetota, with clear decoupling between microbial abundance and ARG contribution. Compared with birds, *Marmota himalayana* presented distinct microbiome and resistome structures, with Bacillota acting as the dominant ARG-associated phylum.

Conclusions

These findings reveal host- and environment-associated variation in the gut microbiomes and resistomes of plateau wildlife and highlight wild birds and sympatric mammals as potential reservoirs of clinically relevant ARGs in alpine ecosystems.

1 Background

Antimicrobial resistance (AMR) has been recognized by the World Health Organization (WHO) as one of the most critical public health challenges of the 21st century [1]. In 2019, antibiotic-resistant bacterial infections were associated with 4.95 million deaths worldwide [2], and the number of annual deaths attributable to antimicrobial resistance is projected to reach 10 million by 2050 [3, 4], surpassing cancer-related mortality and resulting in cumulative global economic losses exceeding USD 100 trillion [5]. Critically, the persistent emergence of multidrug-resistant (MDR) bacteria has further exacerbated the disease burden associated with AMR-related infections, which have become among the leading causes of mortality worldwide, surpassing several well-recognized causes of death, such as HIV/AIDS [6].

Antibiotic resistance genes (ARGs) are a class of emerging environmental contaminants, facilitating their continuous spread in aquatic environments, soils, and biota, thereby posing serious threats to ecological security and public health. In particular, the reported plasmid-mediated colistin resistance gene *mcr-1* signals a potential breach of last-resort antibiotics [7]. China is among the largest producers and consumers of antibiotics worldwide, with an estimated annual usage of 150,000–200,000 tons, approximately 10-fold higher than that in the United States [8]. Approximately half of these antibiotics are used in human medicine, with the remainder applied in animal husbandry. This extensive use has been implicated in the emergence and dissemination of antimicrobial resistance, posing a substantial threat to global public health.

Wildlife serve as significant sources and reservoirs of ARGs, with invertebrates [9], birds [10] and mammals [11, 12] contributing to their dissemination. Among these, wild birds are particularly notable because of their long-distance migratory capabilities, diverse feeding habits, substantial overlap with areas of human activity and recognized role as profound biological vectors connecting the environment, livestock, and human populations [13]. By facilitating the transboundary and remote dissemination of antibiotic-resistant bacteria, wild birds may further exacerbate the global spread of antimicrobial resistance [11]. For example, migratory birds carrying the *mcr-1* gene have been reported across multiple countries and regions, including China [14], Argentina [15], Russia [16] and Thailand [17], following dissemination from Asia. Recent studies have further demonstrated that wild birds harbor diverse and abundant ARGs. Quantitative analyses have revealed that the absolute abundance of ARGs in the intestinal tract of wild birds can reach 10–10¹⁰ copies/g [18], significantly exceeding that detected in environmental matrices such as soil, sediment, and water, while showing no statistically significant difference compared with the abundance of ARGs in livestock and poultry feces. ARGs have been identified in more than 30 avian species belonging to multiple orders, including Charadriiformes [19], Pelecaniformes [20], Anseriformes [21], Columbiformes [22], Passeriformes, and Ciconiiformes [23]. Notably, one study reported that the prevalence of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* in gulls (*Larus canus*) from the same geographic region was more than three times greater than that observed in humans [24]. Compared with abiotic dissemination pathways such as water flow and atmospheric transport, biologically mediated ARG transmission through wildlife and food webs may exhibit greater persistence and ecological connectivity, thereby facilitating the widespread environmental exchange and dissemination of ARGs [25, 26].

The Qinghai–Tibet Plateau has long been regarded as a relatively pristine ecosystem with limited anthropogenic disturbance; however, increasing evidence suggests that this region also harbors potential risks associated with antimicrobial resistance dissemination. As the highest-elevation ecosystem in the world, the Qinghai–Tibet Plateau is characterized by hypoxia, low temperatures, and intense ultraviolet radiation, which together shape unique biological and microbial community structures [26, 27]. With the development of high-throughput sequencing technologies, recent studies have revealed the remarkable complexity of resistomes in plateau wildlife. Metagenomic analyses demonstrated that despite inhabiting relatively remote environments, wild birds on the Qinghai–Tibet Plateau harbor diverse and abundant ARGs within their gut microbiomes, reflecting the potential influence of anthropogenic

activities on these originally isolated ecosystems. For example, a total of 20 ARG classes and 567 ARG subtypes were identified in five corvid bird species, among which multidrug resistance genes and resistance determinants associated with macrolide–lincosamide–streptogramin (MLS), tetracycline, β -lactam, and bacitracin antibiotics were highly abundant [28]. In addition, broad-spectrum resistance-associated genes have been identified in microorganisms associated with *Himalayan vultures* [29] and Tibetan yaks [30] on the Qinghai–Tibet Plateau, suggesting that plateau ecosystems may already function as important environmental reservoirs of ARGs. Because wild birds exhibit broad movement ranges, long-distance migration, and frequent excretion behaviors, they may facilitate the dissemination of ARGs among natural environments, water systems, and human-associated habitats and may even contribute to interregional transmission. Therefore, systematically characterizing the resistome profiles of the wild birds inhabiting the Qinghai–Tibet Plateau is highly important for understanding the ecological dissemination mechanisms of antimicrobial resistance (AMR) and for developing effective surveillance and control strategies.

2 Methods

2.1 Sampling

Sampling efforts were carried out at three sites in Tianjun County, Menyuan County and Xinghai County, Qinghai Province, from July to August 2024 (Table S1). We trapped wild birds using live trapping, locked them in sterilized cages (typically for less than 30 min) until defecation, and then immediately released them at the capture site; additionally, Himalayan marmots (*Marmota himalayana*, Marmota) were captured and euthanized for the collection of their intestinal contents. Fresh feces were collected in 2-mL tubes (Kejin, China) and immediately frozen in liquid nitrogen before being sent for analysis to the Qinghai Provincial Institute for Endemic Disease Prevention and Control, Xining, China. The fecal samples were collected at a minimum distance interval of five meters to ensure that all the fresh droppings were expelled from different individuals, and the captured individuals were marked with nontoxic avian leg bands prior to release. All the samples were immediately placed in sterile 50-mL tubes, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent DNA analysis. In total, we collected 27 bird fecal samples, of which 7 were from Hodgson's Redstart (*Phoenicurus hodgsoni*, PH), 4 from Eurasian Tree Sparrow (*Passer montanus*, PM), 4 from Rosy Pipit (*Anthus roseatus*, AR), 4 from Eurasian Skylark (*Alauda arvensis*, AA), 4 from Tibetan Snowfinch (*Montifringilla henrici*, MH), and 4 from Northern Wheatear (*Oenanthe oenanthe*, OO). In addition, we collected feces from the same region where three *Marmota himalayana* were trapped.

2.2 DNA extraction and sequencing

Metagenomic DNA was isolated from approximately 0.25 g samples using a TIANamp Soil DNA Kit (Tiangen Biotech; Beijing; China) following the manufacturer's instructions. The DNA concentration and quality were assessed with an Agilent 5,400 instrument. High-quality DNA was randomly fragmented into segments of approximately 350 bp using a Covaris ultrasonic disruptor to construct the library. Using the

Rapid Plus DNA Lib Prep Kit for Illumina (RK20208), sequencing libraries were constructed through end repair, the addition of A-tails, the ligation of sequencing adapters, purification, and PCR amplification. All the libraries were then subjected to 2 x 150 bp paired-end sequencing on the BGISEQ DNBSEQ-T7 platform (Novogene, Beijing, China).

2.3 Sequence analyses and metagenome assembly

The raw BGISEQ paired-end sequencing data were preprocessed using Fastp (v0.23.1). The preprocessing pipeline consisted of adapter trimming, followed by quality filtering. During the filtering step, read pairs were discarded if either read contained (a) residual adapter sequences; (b) > 10% ambiguous bases (N); or (c) > 50% low-quality bases (Phred score < 5). Considering the possibility of host contamination in samples, the clean data were subjected to BLAST analysis against the host database to filter out reads of host origin. Bowtie2 software (v2.5.4) was used with the following default parameter settings: --end-to-end, --sensitive, -I 200, and -X 400 [31–33]. MEGAHIT software (v1.2.9) was used for assembly analysis of the clean data, with the following assembly parameter settings: --presets meta-large (--end-to-end, --sensitive, -I 200, -X 400) [32, 34], and scaftigs without N were obtained by breaking the resulting scaffolds from the N junction [35, 36].

2.4 Gene prediction and construction of the nonredundant gene set

Using the default parameters, MetaGeneMark (v2.1) was used to predict the ORF for the scaftigs (≥ 500 bp) of each sample [37–40], and sequences with a length of less than 100 nt in the prediction results were filtered out [41–43]. For the ORF prediction results, CD-HIT software (v4.5.8) was used to eliminate redundancy [44, 45] and obtain the nonredundant initial gene catalog (representing the nucleotide sequences of successive, nonredundant genes) [42], with the following parameter settings: -c 0.95, -G 0, -aS 0.9, -g 1, and -d 0 [38, 40]. Clean data from each sample were aligned to the initial gene catalog by using Bowtie2 to calculate the number of reads of the genes in each sample alignment, with the following parameter settings: --end-to-end, --sensitive, -I 200, and -x 400 [35, 38]. Genes with ≤ 2 reads in each sample were filtered out to finally determine the gene catalog (unigenes) for subsequent analysis [42]. On the basis of the abundance of each gene in the gene catalog in each sample, the abundance information of each unigene in each sample was statistically analyzed. To ensure accurate characterization of the prokaryotic microbiome, all sequences taxonomically assigned to eukaryotes and viruses were excluded from downstream analyses, including taxonomic profiling, functional annotation, and antibiotic resistance gene screening.

2.5 Gene taxonomic prediction

DIAMOND software (v2.1.9) [46] was used for alignment of unigene sequences with the sequences in the Micro_NR database, which included sequences from bacteria, fungi, archaea, and viruses extracted from the NCBI NR database (<https://www.ncbi.nlm.nih.gov/>). The alignment was performed using the BLASTP algorithm with a parameter setting of $1e-5$ [32]. From the alignment results of each sequence, the one with an e-value $\leq \min$. An e-value $\times 10$ was selected. Since each sequence may have multiple

alignment results, the lowest common ancestor (LCA) algorithm [applied to the systematic taxonomy of MEGAN software (https://en.wikipedia.org/wiki/Lowest_common_ancestor)] was adopted to determine the species annotation information of the sequence [47]. In addition to the results of the lowest common ancestor (LCA) annotation and gene abundance table, the abundance of each sample at each taxonomic level and the corresponding gene abundance tables were acquired. The abundance of a species in a sample was equal to the sum of the abundance of those genes annotated as that species. Microbiome analyses were conducted using a standardized bioinformatics pipeline. Taxonomic abundance profiles across hierarchical levels were visualized through relative abundance profiles and a Bray–Curtis distance-based clustering heatmap implemented in Perl SVG (v5.18.2).

2.6 Functional gene annotation

Functional annotation of the unigenes was performed using DIAMOND (v2.1.9) with BLASTP alignment against the Kyoto Encyclopedia of Genes and Genomes (KEGG) (v2023.1) [48, 49] and Pathogen–Host Interaction (PHI) database (v4.12) by applying an e-value cutoff of $1e-5$ [38, 50]. From the alignment results of each sequence, the best BLAST hit results were selected for subsequent analysis [51, 52]. The relative abundance at different functional levels was calculated according to the alignment results (the relative abundance at each functional level was equal to the sum of the relative abundance of genes annotated at that functional level) [31, 38]. For antibiotic resistance profiling, the unigenes were aligned to the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>) [53] using Resistance Gene Identifier (RGI) software (v6.0.2) [54] provided by the CARD database (RGI built-in BLASTP, default e-value $< 1e-30$) [55]. The relative abundance of each ARO was calculated according to the RGI alignment results and unigene abundance information. To reveal the relationship between the microbial composition and the resistome, Circos (v0.64) was used to visualize the abundance distribution circle map. Resistance genes (unigenes annotated as ARO) and species attribution analysis of the resistance mechanism were carried out (some AROs with long names are abbreviated as the first three words plus underlines).

2.7 Statistical analysis

Venn diagrams were generated using the R package Venn Diagram (v3.0.3). The alpha diversity, including the ACE, Chao1 richness, observed_species, Shannon and Simpson indices, was computed using the R package vegan (v2.15.3). Differences in alpha diversity between groups were assessed using the nonparametric Kruskal–Wallis test. Beta diversity was evaluated on the basis of Bray–Curtis dissimilarity matrices, and group differences were tested using permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations via the `adonis2` function in the vegan package. Principal coordinate analysis (PCoA) was performed to visualize beta diversity patterns using the R packages `extrafont`, `ggplot2`, and `grid` (v2.15.3). LEfSe software (v1.0) was used for the linear discriminant analysis effect size (LEfSe) analysis (LDA score was 4 by default) [56]. Statistical significance was set at $p < 0.05$.

3 Results

3.1 Distinct gut bacterial communities in different wild bird populations

Compared with archaea, eukaryotes, and viruses, bacterial units were predominant in all bird species and contributed more phylogenetic diversity (Supplementary File 1). At the phylum level, the sequenced reads from all the samples assigned to the top 10 different phyla and bird species differed markedly in their patterns of bacterial composition (Fig. 1a; Supplementary File 2). In the PH, MH and OO sample groups, the most abundant phylum was *Actinomycetota* (34.42%, 36.98%, and 40.66%, respectively), whereas in the AR and AA groups, the most enriched phylum was *Pseudomonadota* (57.87% and 39.96%, respectively). The proportion of *Bacillota* (56.40%) was greater in the PM group. In general, *Pseudomonadota*, *Actinomycetota*, *Bacillota*, and *Chlamydiota* were the four dominant phyla in all the species, accounting for 10.99–32.78% of the total bacterial community. At the genus level, distinct differences in dominant taxa were observed among the groups (Figure S1; Supplementary File 3). The PH group was dominated primarily by *Mycobacterium* and *Chlamydia*, whereas *Lactococcus*, *Saccharopolyspora* and *Escherichia* were the predominant genera in the PM group. In the PM group, *Lactococcus* was the most abundant genus, followed by *Arthrobacter*, *Rhodococcus*, *Rhabdochlamydia*, and *Chlamydia*, whose abundance was relatively high. The AA group was characterized by enrichment of *Enterococcus* and *Rhabdochlamydia*. In the MH group, *Brachyspira*, *Rhodococcus* and *Enterococcus* were the most abundant genera, whereas the OO group was dominated mainly by *Chlamydia*, *Mycobacterium* and *Rhodococcus*. In addition, the relatively high proportion of “Others” across all groups suggests a considerable level of microbial diversity within the samples. The alpha diversity analysis revealed that the Chao1, observed species, Shannon, and Simpson indices were 34.32 ± 26.15 , 33.3 ± 25.59 , 0.86 ± 0.40 , and 0.41 ± 0.22 , respectively, with significant differences observed in the Shannon ($P = 0.0077$) and Simpson ($P = 0.0067$) indices among the groups (Fig. 1b and c). Similarly, the beta diversity analysis further demonstrated differences in microbial community composition among species. Both the heatmap (Fig. 2a) and principal coordinate analysis (PCoA) based on Bray–Curtis distances (Fig. 2b) revealed clear dissimilarities in bacterial communities (PERMANOVA, $R^2 = 0.27$, $P = 0.016$). Interestingly, samples from species inhabiting the same geographic environment (i.e., the PM, AR and AA groups from Menyuan County) tended to cluster together, suggesting that environmental factors may play a more important role than host genetic background in shaping the gut microbiota. A Venn diagram revealed that a total of 35 taxa were shared across all groups, indicating a conserved core microbiota, whereas 7–75 unique taxa were identified in each group, indicating group-specific microbial features (Figure S2). LEfSe analysis was subsequently performed to identify differentially abundant taxa among groups. No significant differences were detected at the phylum level. Several taxa were significantly enriched in specific groups at the genus level (Fig. 2c). Specifically, *Malacoplasma* was enriched in the PM group; the abundance of *Mycobacterium*, *Salmonella* and *Bacillus* was greater in the PH group; *Chlamydia* and *Aeromonas* were dominant in the OO group; and *Catellibacillus* was significantly enriched in the AA group. These findings indicate distinct microbial signatures among groups and suggest that intergroup differences are more pronounced at finer taxonomic resolution.

On the basis of the PHI database annotation, a total of 202 opportunistic pathogenic species were identified across all the samples (Supplementary File 4). Among these, several clinically important pathogens, including *Staphylococcus aureus*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumoniae*, were the most prevalent taxa. In addition, zoonotic and high-risk pathogens such as *Yersinia pestis*, *Vibrio cholerae*, *Brucella spp.* and *Coxiella burnetii* were also identified, indicating potential public health risks.

3.2 Functional divergence of the gut microbiome of wild birds

We then annotated the metabolic and functional pathways of the birds' gut microbiome nonredundant (NR) gene catalog based on the KEGG database. Approximately 32% of the NR genes (8,145 KEGG Orthology (KO) functions) were mapped to 396 KEGG level 3 pathways and were subsequently classified into 45 secondary KEGG level 2 pathways. The unigenes matching the level 1 and level 2 KEGG functional categories are shown in Fig. 3. Specifically, the dominant functional categories identified included metabolism (44.64%), genetic information processing (16.71%), and environmental information processing (13.64%) (Fig. 4a).

We compared α diversity (ACE, Chao1, observed species, Shannon, and Simpson indices) among individuals at the 1–3 level and at the KO level. Since no significant differences were observed in the α diversity, we further assessed functional variation using the β -diversity analysis results. PCoA revealed clear clustering among the groups (Fig. 4b). Interestingly, samples from the PM, AR, and AA groups (same geographic region) clustered together, whereas those from the MH and OO groups formed a separate cluster. This pattern is consistent with the results of the taxonomic analysis, further supporting the role of environmental filtering in shaping both the taxonomic and functional profiles of the gut microbiota. To further identify the key functional features driving the observed differences, LEfSe analysis was performed on the KEGG level 2 pathways (Fig. 4c). Several pathways were differentially enriched among the groups. Carbohydrate metabolism was significantly enriched in the PM group, whereas pathways related to antimicrobial drug resistance were more abundant in the AR group. The OO group was associated mainly with environmental adaptation and disease-related pathways, including cardiovascular, neurodegenerative, and cancer-related functions. In contrast, the PH group was enriched in endocrine and metabolic disease pathways, whereas the AA group was characterized by enhanced replication and repair functions. These results further support the functional divergence among groups and highlight distinct metabolic and adaptive strategies of the gut microbiome.

3.3 Abundant ARGs in the gut bacteria of wild birds

Annotation against the CARD (v3.2.5) revealed 130 unique ARO categories in the gut microbiome (Supplementary File 5). Among the 15 most abundant ARO genes (Fig. 5a), *rsmA*, *Escherichia coli* EF-Tu mutants conferring resistance to pulvomycin, *vanW* gene in the *vanI* cluster and *vanT* gene in the *vanG* cluster were present in six bird populations, whereas compared with the other groups, the PM, AA and MH sample groups had more ARO genes in common (*mdtE*, *Escherichia coli* *soxR* with mutation

conferring antibiotic resistance, *mdtF*, *Escherichia coli soxS* with mutation conferring antibiotic resistance, *mdtP*, *eptA* and *mdtN*). ARG abundance was highest in the PM group, followed by the AR and AA groups. Most of these genes were associated with clinically important classes of antibiotics, including β -lactams, quinolones, tetracyclines, and polymyxins. Notably, genes related to multidrug efflux systems and colistin resistance (e.g., *eptA*) were detected, indicating the presence of resistance mechanisms against last-resort antibiotics.

The alpha diversity indices (Chao1, observed species, Shannon, and Simpson indices) were 28.87 ± 27.7 , 27.0 ± 26.2 , 1.81 ± 1.20 and 0.70 ± 0.29 , respectively, with no significant differences detected among the species. We next assessed the global similarity of the ARG composition in each population by PCoA. Although a degree of dispersion was observed, no significant differences were detected among the groups (PERMANOVA, $R^2 = 0.1891$, $F = 0.9796$, $P = 0.3996$) (Fig. 5b). These results suggest that the overall ARG composition was relatively similar among the bird groups. To investigate the compositional structure underlying this convergent pattern, we further analyzed the sharing profiles of the detected ARGs. The AA, MH and PM groups dominated the resistome, containing 110, 95 and 93 AROs, respectively, and collectively represented approximately 85% of the total, among which 26 AROs were shared across the six groups (Fig. 5c). Despite these differences in ARO distribution, LEfSe analysis did not reveal any significantly different ARGs among the groups, indicating that the overall resistome composition was largely conserved across bird populations.

Circos plot analysis revealed clear phylogenetic partitioning of antibiotic resistance mechanisms across bacterial phyla (Fig. 6). Among the detected mechanisms, antibiotic target alteration was the most widely distributed and showed extensive associations with multiple dominant phyla, particularly *Bacillota*. Antibiotic efflux was also broadly represented and strongly associated with *Pseudomonadota*. Antibiotic inactivation was distributed across several phyla, including *Bacillota*, *Actinomycetota*, *Pseudomonadota* and *Bacteroidota*, whereas minor phyla contributed only marginally to all resistance categories. Overall, these results indicate that antibiotic resistance mechanisms were unevenly distributed among bacterial phyla, with *Bacillota* and *Pseudomonadota* representing the primary reservoirs of major resistance determinants, including those of potential clinical relevance.

The microbial origins of the ARGs were further investigated (Fig. 7a-f). *Pseudomonadota*, *Bacillota* and *Actinomycetota* accounted for the majority of the phyla associated with resistance-conferring contigs, although their relative contributions varied among groups. *Pseudomonadota* was the dominant phylum across all groups, contributing approximately 31% to the PH group, 56% to the PM group, 68% to the AR and AA groups, 41% to the MH group, and 46% to the OO group. *Bacillota* contributed relatively more to the PH (29%), AA (20%), and MH (28%) groups, whereas *Actinomycetota* contributed substantially to the PM (16%), AR (13%), and OO (26%) groups. PH exhibited a relatively balanced distribution among the three major phyla (31%, 29%, and 25%). Discrepancies between microbial abundance and ARG contribution were evident. In several cases, relatively low-abundance phyla contributed disproportionately to the ARGs. For example, in the OO group, *Pseudomonadota* accounted for only 2% of the microbial community but contributed 46% of the ARGs. Similar patterns were observed for

Actinomycetota in the AR and OO groups and *Bacillota* in the PH and MH groups, whose ARG contributions were greater than expected on the basis of their relative abundance. These findings indicate that ARG distribution is not directly proportional to taxonomic abundance, suggesting that specific phyla may act as key reservoirs of resistance genes despite their low representation in the microbial community.

3.4 Comparison of gut microbiomes and resistomes between birds and *Marmota himalayana*

We compared metagenomic sequencing data from wild bird feces with those from the sympatric wild rodent *Marmota himalayana* to evaluate whether host-specific selection shapes the ARG distribution beyond environmental filtering. The bacterial community composition at the phylum level differed markedly between hosts (Fig. 8a; Supplementary File 6). The PCoA results indicated that the microbial structure of birds differed from that of *Marmota himalayana* in terms of PC1 variation (Fig. 8b). The phylogenetic composition of the *Marmota himalayana* gut microbiota was less heterogeneous than that of the bird microbiota, whereas the bird-associated microbiota was more dispersed, likely reflecting broader ecological heterogeneity among avian samples. Similarly, the resistome composition differed between hosts and was separated primarily along PC1 according to the PCoA results (Fig. 8c). Compared with those in birds, the ARG profiles in *Marmota himalayana* were more clustered, suggesting a relatively stable resistome structure within marmot populations. In contrast, the ARG compositions of the bird samples were substantially more variable, which is consistent with their more diverse microbial communities and broader environmental exposure. They shared 60 antibiotic resistance proteins (Fig. 8d), predominantly vancomycin resistance-associated genes, including *vanW*, *vanT*, *vanY*, and members of the *vanG* gene cluster, indicating the widespread distribution of glycopeptide resistance determinants among sympatric wildlife hosts. The diversity of the unique ARGs was greater in birds. The marmot-specific resistome contained several clinically relevant ARGs, including nitroimidazole resistance genes (*nimA*, *nimB*, *nimC*, *nimE*, *nimH*, *nimJ*, and *nimK*), tetracycline resistance genes (*tet(Q)* and *tet(36)*), and the oxazolidinone-phenicol resistance gene *poxA*. In addition, multiple vancomycin resistance-associated genes, including *vanH*, *vanR*, *vanW*, *vanXY* and *vanX* variants, were uniquely detected in *Marmota himalayana*. These findings suggest that *Marmota himalayana* may act as a potential reservoir for clinically important ARGs in alpine ecosystems. To further characterize resistome distribution patterns across hosts, a double-circle plot was constructed to depict the microbial origins of ARGs in *Marmota himalayana* (Fig. 8e). *Bacillota* was the dominant contributor, accounting for 85% of the detected ARGs while comprising 62% of the microbial community. In contrast, *Bacteroidota* represented 17% of the microbiota but contributed only 4% of the ARGs. *Pseudomonadota* contributed disproportionately to the ARGs, accounting for 3% of the ARGs despite representing only 1% of the community. Other phyla, including *Actinomycetota* and *Verrucomicrobiota*, contributed negligibly to the overall ARG pool.

4 Discussion

In this study, the microbiomes and resistomes of 6 bird species, namely, *Phoenicurus hodgsoni* (seven samples), *Passer montanus* (four samples), *Anthus roseatus* (four samples), *Alauda arvensis* (four samples), *Montifringilla henrici* (four samples) and *Oenanthe oenanthe* (four samples), were explored by metagenomic sequencing. Our taxonomic analysis revealed substantial variation in the gut microbial composition among the six avian groups, despite the shared presence of several dominant phyla. *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Chlamydiota* were consistently detected across all the groups, which is consistent with previous studies that have identified these taxa as core components of the avian gut microbiota [57]. However, the relative abundances of these phyla differed markedly among species, suggesting that host- and environment-specific factors shape microbial community structure. As most of the members of *Actinomycetota* are widespread in soil, water, or air, these bacteria in birds may have originated from the environment, suggesting that the bird gut microbiota is also influenced by bacterial transmission [58]. With the help of *Bacillota*, animal hosts can intake energy and absorb nutrients from the metabolism of carbohydrates, polysaccharides, sugars, and fatty acids [59]. At the genus level, the gut microbiota of the six plateau bird groups exhibited both shared characteristics and pronounced group-specific signatures. *Chlamydia*, *Mycobacterium*, and *Rhodococcus* were frequently detected across multiple groups, suggesting that these genera may represent environmentally persistent or host-adapted components of the gut microbiota in high-altitude wild birds. *Chlamydia* has been widely reported in wild avian populations and is recognized as an important zoonotic genus associated with avian chlamydiosis, particularly *C. psittaci*, which is broadly distributed among migratory and free-living birds [60, 61]. As obligate intracellular bacteria, *Chlamydia* species utilize a type III secretion system (T3SS) to manipulate host cellular processes and evade immune clearance, thereby facilitating their persistence and transmission in wild bird populations. In addition, several *Chlamydia* species are important human pathogens associated with respiratory, ocular, and gastrointestinal diseases [62, 63]. Similarly, *Mycobacterium* and *Rhodococcus* are widely distributed in natural environments, including soil and water, and are commonly detected in wild birds [64, 65]. Environmental *Mycobacterium* species are characterized by strong persistence and resistance to harsh conditions, which may contribute to their maintenance in plateau ecosystems. Their occurrence across several bird groups may therefore reflect continuous environmental exposure under shared ecological conditions. Taxonomic variation in the gut microbiota among the six plateau bird groups was more pronounced at the genus level than at higher taxonomic ranks. Although no significant differences were detected at the phylum level, LEfSe analysis revealed multiple group-enriched genera, highlighting the greater sensitivity of finer taxonomic resolution for detecting host-associated microbial variation. Consistently, significant differences in alpha diversity, together with the clear separation observed in PCoA, indicated substantial heterogeneity in microbial community structure among host groups. Notably, birds from the same geographic region tended to cluster together, suggesting that shared habitat conditions may play an important role in shaping gut microbiota assembly, which is consistent with the findings of previous studies in wild birds [66, 67]. We further gained insight into the microbiological risks in wild birds. A total of 202 detected genera contain opportunistic or zoonotic pathogens, underscoring the potential ecological and public health significance of wild birds as reservoirs of environmentally persistent microorganisms. Previous studies have demonstrated that migratory and free-living birds can facilitate the environmental dissemination

and long-distance transmission of pathogenic microorganisms, thereby contributing to potential ecological and public health risks. However, because metagenomic annotation alone cannot distinguish active infection from transient environmental carriage, further culture-based and functional studies are needed to clarify the pathogenic potential and transmission risks associated with these microorganisms.

ARGs can readily enter the avian gut from the environment through feeding and other ecological activities, thereby making birds important reservoirs of antibiotic-resistant bacteria and resistance genes. These birds may subsequently mediate the release of ARGs back into the environment through excretion and related activities, ultimately forming an “environment–bird–environment” ARG interaction network. In the present study, a total of 130 unique ARO categories were identified across all the birds. Multidrug resistance-associated genes, as well as resistance determinants related to clinically important antibiotic classes, including β -lactams, quinolones, tetracyclines, and polymyxins, were widely distributed. Among them, resistance-associated genes such as *rsmA*, *vanW*, and *vanT* were detected in all the bird groups, suggesting the presence of a relatively conserved core resistome structure among plateau bird populations. The gulls have been found to be reservoirs of beta-lactam resistance in Europe, especially in Spain [68]. The detection of *eptA* and multiple efflux pump-associated genes suggests the presence of resistance mechanisms against the last-resort antibiotic colistin within the gut microbiota of plateau wild birds. Colistin resistance genes have attracted increasing attention because polymyxins are considered critical antibiotics for treating infections caused by multidrug-resistant bacteria [69]. *EptA* encodes a phosphoethanolamine transferase that mediates the modification of lipid A through the addition of phosphoethanolamine, thereby reducing the binding affinity of polymyxins such as colistin to the bacterial outer membrane. Recent studies have further shown that endogenous phosphoethanolamine transferases such as *eptA* may represent evolutionarily conserved precursors of transferable *mcr* genes, highlighting a potential evolutionary link between intrinsic and mobile colistin resistance mechanisms [70]. In addition to mediating resistance, *eptA*-associated lipid A modification may also increase bacterial membrane stability and environmental adaptability under stressful ecological conditions. Cervoni et al. [71] demonstrated that phosphoethanolamine transferases can differentially affect bacterial fitness and membrane physiology, suggesting that the maintenance of *eptA* may provide ecological advantages beyond antibiotic resistance alone [71, 72]. Among the six bird groups, *Oenanthe oenanthe* (OO) was the only long-distance migratory species that primarily feeds on insects, whereas the other five species utilize multiple food resources; however, it exhibited the lowest ARG richness. Compared with omnivorous birds, which frequently forage in agricultural or human-associated habitats, *Oenanthe oenanthe* mainly inhabit relatively pristine environments during breeding and migration, which may reduce their exposure to anthropogenic antibiotic residues and resistant microorganisms. In addition, their insectivorous feeding strategy may further limit ARG acquisition through contaminated food and water sources. Collectively, these findings indicate that compared with migratory behavior itself, habitat quality and feeding ecology may play more important roles in shaping ARG carriage in wild birds. Moreover, compared with birds from other regions, bird groups inhabiting Menyuan County presented relatively higher ARG abundance and greater numbers of ARGs. Previous

studies have demonstrated that environmental factors, including anthropogenic activity, livestock farming, wastewater contamination, and habitat overlap, can substantially influence ARG dissemination in wildlife-associated microbiomes [26, 73]. Compared with more remote plateau regions, Menyuan County is characterized by intensive animal husbandry and relatively frequent human activities, which may increase environmental antibiotic residues and resistant bacterial transmission through soil, water, and food sources. In addition, sympatric bird species that share similar habitats and feeding environments may facilitate microbial exchange and ARG circulation among hosts [25]. Regrettably, because environmental samples were not included in the present study, the direct contribution of local environmental reservoirs to ARG enrichment could not be confirmed. Therefore, further studies integrating environmental microbiome sampling and anthropogenic exposure assessments are needed to clarify the ecological drivers underlying regional ARG variation in plateau birds.

Most of the ARGs (78 out of 60) detected in local rodents were also detected in wild birds, and the relative abundance of these shared ARGs was generally higher in birds. This substantial overlap suggests the presence of common environmental reservoirs or shared exposure routes between the two host groups. The coutilization of water sources and grazing habitats on the Qinghai–Tibet Plateau, together with the burrow-associated nesting behaviors of both birds and rodents, may facilitate indirect microbial exchange through environmental contact. Despite the shared ARG pool, the consistently higher abundance of these genes in birds implies that birds might act as net donors or amplifiers of resistance determinants, whereas rodents serve as passive recipients. This pattern aligns with the hypothesis that long-distance migratory birds, which are exposed to diverse anthropogenic antibiotic pressures along their flyways, can carry enriched ARGs and subsequently disseminate them to sedentary local wildlife via fecal-oral or environmental routes. However, establishing the directionality of ARG transmission (i.e., from birds to rodents) would require additional evidence, such as longitudinal sampling, shared mobile genetic element analysis, or plasmid-typing, and alternative interpretations (e.g., a common environmental source) cannot be ruled out. In birds, ARGs were associated mainly with *Pseudomonadota*, *Bacillota*, and *Actinomycetota*, with *Pseudomonadota* acting as the major contributor in most groups. In contrast, the Himalayan marmot resistome was overwhelmingly dominated by *Bacillota*. A decoupling between microbial abundance and ARG contribution was observed across hosts. In birds, several low-abundance phyla, including *Pseudomonadota*, *Bacillota*, and *Actinomycetota*, contributed disproportionately to ARGs in specific groups. Similarly, in the Himalayan marmot, *Pseudomonadota* contributed 3% of the ARGs, despite accounting for only 1% of the microbiota, whereas *Bacteroidota* showed the opposite pattern, representing 17% of the microbiota but contributing only 4% of the ARGs. Overall, these results indicate that ARG carriage is not simply determined by microbial abundance. Instead, specific bacterial phyla act as key ARG reservoirs in a host-dependent manner, with *Pseudomonadota* predominating in birds and *Bacillota* predominating in the Himalayan marmot. These findings highlight the importance of host identity in the structuring of ARG reservoirs and suggest that wildlife species may differ substantially in their potential roles in maintaining and disseminating resistance determinants in alpine ecosystems.

Despite these findings, several limitations should be acknowledged. First, the sample size for each bird group and for *Marmota himalayana* was relatively limited, which may have constrained the statistical power for detecting subtle host-associated differences. Second, environmental samples, such as soil, water, and food resources, were not included in this study, preventing direct evaluation of environmental contributions to microbiome assembly and ARG dissemination. Third, metagenomic sequencing can be used to identify the presence of ARGs but cannot determine their transcriptional activity, phenotypic resistance, or horizontal transfer potential. In addition, the short-read sequencing strategy used here limited the resolution of the ARG genomic context and mobile genetic elements. Therefore, future studies that integrate environmental sampling, culture-based isolation, quantitative PCR, metatranscriptomics, and long-read sequencing approaches will be necessary to better characterize the activity, mobility, and ecological transmission dynamics of ARGs in plateau wildlife ecosystems.

5 Conclusions

This study provides a metagenomic overview of the gut microbiomes and resistomes of six wild bird species inhabiting the Qinghai–Tibet Plateau and compares them with those of sympatric Himalayan marmots. The results revealed marked variation in gut microbial composition among bird groups, whereas birds from the same geographic region presented more similar taxonomic and functional profiles, suggesting that local environmental conditions contribute substantially to microbiome assembly. A diverse resistome was identified in wild birds, including resistance determinants associated with multidrug resistance and clinically important antibiotic classes. Although the overall ARG composition was relatively conserved among the bird groups, the microbial origins of the ARGs varied substantially, indicating that ARG carriage was not simply determined by bacterial abundance. Comparative analysis further demonstrated host-associated differences in the microbiome and resistome structure between birds and *Marmota himalayana*, with Pseudomonadota predominating as ARG reservoirs in birds and Bacillota predominating in marmots. Overall, these findings highlight plateau wildlife as potential reservoirs and disseminators of clinically relevant ARGs and provide new insights into the ecological dissemination of antimicrobial resistance in alpine ecosystems.

Abbreviations

Abbreviation	Full Form
AA	<i>Alauda arvensis</i> (Eurasian Skylark)
AMR	Antimicrobial Resistance
AR	<i>Anthus roseatus</i> (Rosy Pipit)
ARGs	Antibiotic Resistance Genes
AROs	Antibiotic Resistance Ontology (CARD categories)
CARD	Comprehensive Antibiotic Resistance Database
ESBL	Extended-Spectrum β -Lactamase
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	KEGG Orthology
LCA	Lowest Common Ancestor
LDA	Linear Discriminant Analysis
LEfSe	Linear Discriminant Analysis Effect Size
MDR	Multidrug-Resistant / Multidrug Resistance
MH	<i>Montifringilla henrici</i> (Tibetan Snowfinch)
MLS	Macrolide–Lincosamide–Streptogramin
NR	Nonredundant
OO	<i>Oenanthe oenanthe</i> (Northern Wheatear)
ORF	Open Reading Frame
PCoA	Principal Coordinate Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
PH	<i>Phoenicurus hodgsoni</i> (Hodgson's Redstart)
PHI	Pathogen–Host Interaction (database)
PM	<i>Passer montanus</i> (Eurasian Tree Sparrow)
RGI	Resistance Gene Identifier
T3SS	Type III Secretion System
WHO	World Health Organization

Declarations

Ethics approval and consent to participate

The related animal experiments were approved by the Laboratory Animal Management and Ethics Committee of Qinghai Institute for Endemic Disease Prevention and Control (approval number: SYXK (Qing) 2021-0003, approved on March 17, 2023). The study was conducted in accordance with the local legislation and institutional requirements.

Consent for publication

Not applicable.

Availability of data and material

All the data and materials are available upon request.

Competing interests

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

HX: Conceptualization, Resources, Project administration, Funding acquisition, Investigation, Supervision, Methodology, Writing—review & editing. SS: Writing—original draft, Methodology, Investigation, Software, Conceptualization, Data curation, Formal analysis, Writing—review & editing, Visualization. JQ: Investigation, Writing—original draft, Software, Writing—review & editing, Methodology, Visualization. XS: Conceptualization, Writing—review & editing, Formal analysis, Writing—original draft, Methodology, Software. XS: Writing—original draft, Investigation, Formal analysis, Methodology. GA: Data curation, Software, Writing—review & editing, Formal analysis. LM: Software, Resources, Data curation, Writing—original draft. HX: Writing—review & editing, Investigation, Conceptualization. WG: Investigation, Writing—original draft, Data curation. YW: Writing—review & editing, Data curation. PC: Writing—review & editing, Visualization, Validation. YW: Project administration, Writing—review & editing, Investigation,

Supervision, Data curation, Conceptualization, Resources. JY: Project administration, Writing—review & editing, Investigation, Data curation, Conceptualization, Resources. WL: Project administration, Writing—review & editing, Investigation, Supervision, Conceptualization, Resources.

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References

1. Laxminarayan R, Van Boeckel T, Frost I, Kariuki S, Khan EA, Limmathurotsakul D, et al. The lancet infectious diseases commission on antimicrobial resistance: 6 years later. *Lancet Infect Dis*. 2020;20:e51–60.
2. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399:629–55.
3. O'Neill J. Review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations. London, UK: Wellcome Trust; 2016.
4. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. London, UK: Wellcome Trust; 2014.
5. Piddock LJV. Reflecting on the final report of the O'Neill review on antimicrobial resistance. *Lancet Infect Dis*. 2016;16:767–8.
6. Sommer MOA, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science*. 2009;325:1128–31.
7. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16:161–8.
8. Larson C, Pharmaceuticals. China's lakes of pig manure spawn antibiotic resistance. *Science*. 2015;347:704.
9. Zhu D, Xiang Q, Yang XR, Ke X, O'Connor P, Zhu YG. Trophic transfer of antibiotic resistance genes in a soil detritus food chain. *Environ Sci Technol*. 2019;53:7770–81.
10. Marcelino VR, Wille M, Hurt AC, González-Acuña D, Klaassen M, Schlub TE, et al. Meta-transcriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes. *BMC Biol*. 2019;17:31.

11. Rolland RM, Hausfater G, Marshall B, Levy SB. Antibiotic-resistant bacteria in wild primates: increased prevalence in baboons feeding on human refuse. *Appl Environ Microbiol.* 1985;49:791–4.
12. Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi impenetrable national park, Uganda. *Conserv Biol.* 2008;22:1600–7.
13. Guenther S, Aschenbrenner K, Stamm I, Bethe A, Semmler T, Stubbe A, et al. Comparable high rates of extended-spectrum-beta-lactamase-producing *Escherichia coli* in birds of prey from Germany and Mongolia. *PLoS ONE.* 2012;7:e53039.
14. Zhang Y, Kuang X, Liu J, Sun RY, Li XP, Sun J, et al. Identification of the plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* isolates from migratory birds in Guangdong, China. *Front Microbiol.* 2021;12:755233.
15. Liakopoulos A, Mevius DJ, Olsen B, Bonnedahl J. The colistin resistance *mcr-1* gene is going wild. *J Antimicrob Chemother.* 2016;71:2335–6.
16. Tarabai H, Valcek A, Jamborova I, Vazhov SV, Karyakin IV, Raab R, et al. Plasmid-mediated *mcr-1* colistin resistance in *Escherichia coli* from a Black Kite in Russia. *Antimicrob Agents Chemother.* 2019;63:e01266–19.
17. Aeksiri N, Toanan W, Sawikan S, Suwannarit R, Pungpomin P, Khieokhajonkhet A, et al. First detection and genomic insight into *mcr-1* encoding plasmid-mediated colistin-resistance gene in *Escherichia coli* ST101 Isolated from the migratory bird species *Hirundo rustica* in Thailand. *Microb Drug Resist.* 2019;25:1437–42.
18. Zhao H, Sun R, Yu P, Alvarez PJJ. High levels of antibiotic resistance genes and opportunistic pathogenic bacteria indicators in urban wild bird feces. *Environ Pollut.* 2020;266:115200.
19. Atterby C, Ramey AM, Hall GG, Järhult J, Börjesson S, Bonnedahl J. Increased prevalence of antibiotic-resistant *E. coli* in gulls sampled in Southcentral Alaska is associated with urban environments. *Infect Ecol Epidemiol.* 2016;6:32334.
20. Dobbin G, Hariharan H, Daoust PY, Hariharan S, Heaney S, Coles M, et al. Bacterial flora of free-living double-crested cormorant (*Phalacrocorax auritus*) chicks on Prince Edward Island, Canada, with reference to enteric bacteria and antibiotic resistance. *Comp Immunol Microbiol Infect Dis.* 2005;28:71–82.
21. Tsubokura M, Matsumoto A, Otsuki K, Animas SB, Sanekata T. Drug resistance and conjugative R plasmids in *Escherichia coli* strains isolated from migratory waterfowl. *J Wildl Dis.* 1995;31:352–7.
22. Sacristán C, Esperón F, Herrera-León S, Iglesias I, Neves E, Nogal V, et al. Virulence genes, antibiotic resistance and integrons in *Escherichia coli* strains isolated from synanthropic birds from Spain. *Avian Pathol.* 2014;43:172–5.
23. Smith HG, Bean DC, Clarke RH, Loyn R, Larkins JA, Hassell C, et al. Presence and antimicrobial resistance profiles of *Escherichia coli*, *Enterococcus* spp. and *Salmonella* spp. in 12 species of Australian shorebirds and terns. *Zoonoses Public Health.* 2022;69:615–24.

24. Atterby C, Börjesson S, Ny S, Järhult JD, Byfors S, Bonnedahl J. ESBL-producing *Escherichia coli* in Swedish gulls—a case of environmental pollution from humans? PLoS ONE. 2017;12:e0190380.
25. Arnold KE, Williams NJ, Bennett M. Disperse abroad in the land': the role of wildlife in the dissemination of antimicrobial resistance. Biol Lett. 2016;12:20160137.
26. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol. 2010;8:251–9.
27. Liu Y, Priscu JC, Yao T, Vick-Majors TJ, Xu B, Jiao N, et al. Bacterial responses to environmental change on the Tibetan Plateau over the past half century. Environ Microbiol. 2016;18:1930–41.
28. Wang Y, Cui Q, Hou Y, He S, Zhao W, Lancuo Z, et al. Metagenomic insights into the diverse antibiotic resistome of non-migratory corvidae species on the Qinghai-Tibetan Plateau. Vet Sci. 2025;12:297.
29. Zhai J, Wang Y, Tang B, Zheng S, He S, Zhao W, et al. A comparison of antibiotic resistance genes and mobile genetic elements in wild and captive Himalayan vultures. PeerJ. 2024;12:e17710.
30. Wu D, Luo R, Gong G, Zhang L, Huang J, Cai C, et al. Antimicrobial susceptibility and multilocus sequence typing of *Clostridium perfringens* isolated from yaks in Qinghai-Tibet Plateau, China. Front Vet Sci. 2022;9:1022215.
31. Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun. 2012;3:1245.
32. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013;498:99–103.
33. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.
34. Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, et al. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nat Biotechnol. 2014;32:822–8.
35. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65.
36. Li D, Liu CM, Luo R, Sadakane K, Lam TW. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31:1674–6.
37. Mende DR, Waller AS, Sunagawa S, Järvelin AI, Chan MM, Arumugam M, et al. Assessment of metagenomic assembly using simulated next generation sequencing data. PLoS ONE. 2012;7:e31386.
38. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, et al. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol. 2014;32:834–41.
39. Oh J, Byrd AL, Deming C, Conlan S, Kong HH, Segre JA. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014;514:59–64.
40. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature. 2014;513:59–64.

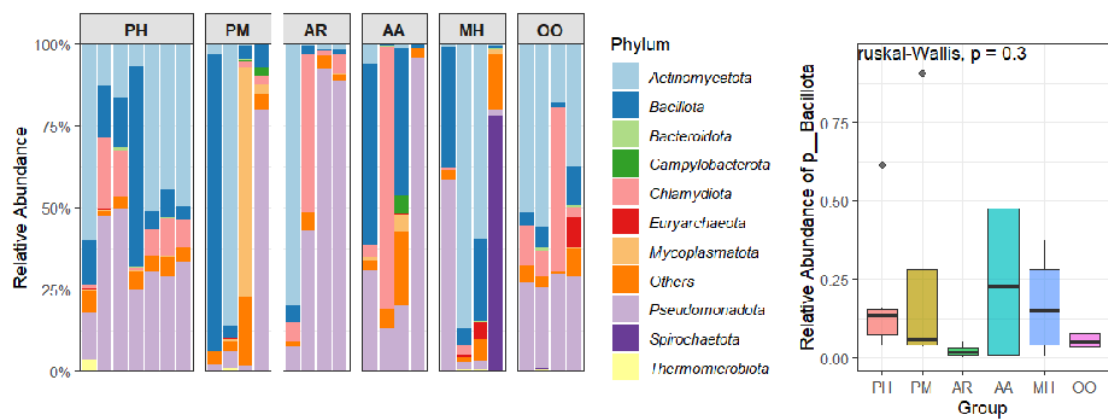
41. Zhu W, Lomsadze A, Borodovsky M. Ab initio gene identification in metagenomic sequences. *Nucleic Acids Res.* 2010;38:e132.
42. Zeller G, Tap J, Voigt AY, Sunagawa S, Kultima JR, Costea PI, et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol.* 2014;10:766.
43. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al. Ocean plankton. Structure and function of the global ocean microbiome. *Science.* 2015;348:1261359.
44. Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics.* 2006;22:1658–9.
45. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics.* 2012;28:3150–2.
46. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. *Nat Methods.* 2015;12:59–60.
47. Huson DH, Mitra S, Ruscheweyh HJ, Weber N, Schuster SC. Integrative analysis of environmental sequences using MEGAN4. *Genome Res.* 2011;21:1552–60.
48. Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, et al. From genomics to chemical genomics: new developments in KEGG. *Nucleic Acids Res.* 2006;34:D354–7.
49. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* 2017;45:D353–61.
50. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun.* 2015;6:6528.
51. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature.* 2012;490:55–60.
52. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe.* 2015;17:852.
53. Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. *Nat Rev Microbiol.* 2015;13:116–23.
54. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 2017;45:D566–73.
55. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother.* 2013;57:3348–57.
56. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 2011;12:R60.
57. Shi S, Qi J, Peng W, Su X, Chen P, Xu S, et al. Convergent gut microbiome adaptation and pervasive antibiotic resistome in Qinghai-Tibet Plateau passerines. *Front Microbiol.* 2026;16:1733974.

58. Mikaelyan A, Dietrich C, Köhler T, Poulsen M, Sillam-Dussès D, Brune A. Diet is the primary determinant of bacterial community structure in the guts of higher termites. *Mol Ecol*. 2015;24:5284–95.
59. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, et al. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol*. 2009;11:2574–84.
60. Stokes HS, Berg ML, Bennett ATD. A review of Chlamydial infections in wild birds. *Pathogens*. 2021;10:948.
61. Wu X, Lei F, Niu Y, Yu J, Chen C, Ba T, et al. Molecular prevalence of *Chlamydia spp.* in wild birds from Qinghai lake, China. *Ir Vet J*. 2025;78:28.
62. Elwell C, Mirrashidi K, Engel J. Chlamydia cell biology and pathogenesis. *Nat Rev Microbiol*. 2016;14:385–400.
63. Wang B, Du P, Huang S, He D, Chen J, Wen X, et al. Comparison of the caecal microbial community structure and physiological indicators of healthy and infection *Eimeria tenella* chickens during peak of oocyst shedding. *Avian Pathol*. 2023;52:51–61.
64. Forbes BA. Mycobacterial taxonomy. *J Clin Microbiol*. 2017;55:380–3.
65. Vazquez-Boland JA, Val-Calvo J, Duquesne F, Decorosi F, Viti C, Petry S, et al. *Rhodococcus parequi* sp. nov., a new species isolated from equine farm soil closely related to the pathogen *Rhodococcus equi*. *Int J Syst Evol Microbiol*. 2025;75:006679.
66. Hird SM, Sánchez C, Carstens BC, Brumfield RT. Comparative gut microbiota of 59 neotropical bird species. *Front Microbiol*. 2015;6:1403.
67. Grond K, Sandercock BK, Jumpponen A, Zeglin LH. The avian gut microbiota: community, physiology and function in wild birds. *J Avian Biol*. 2017;49:e01788.
68. Jarma D, Sánchez MI, Green AJ, Peralta-Sánchez JM, Hortas F, Sánchez-Melsió A, et al. Faecal microbiota and antibiotic resistance genes in migratory waterbirds with contrasting habitat use. *Sci Total Environ*. 2021;783:146872.
69. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev*. 2017;30:557–96.
70. Xu Y, Wei W, Lei S, Lin J, Srinivas S, Feng Y. An evolutionarily conserved mechanism for intrinsic and transferable polymyxin resistance. *mBio*. 2018;9:e02317–17.
71. Cervoni M, Lo Sciuto A, Bianchini C, Mancone C, Imperi F. Exogenous and endogenous phosphoethanolamine transferases differently affect colistin resistance and fitness in *Pseudomonas aeruginosa*. *Front Microbiol*. 2021;12:778968.
72. Shahzad S, Willcox MDP, Rayamajhee B. A review of resistance to polymyxins and evolving mobile colistin resistance gene (mcr) among pathogens of clinical significance. *Antibiot (Basel)*. 2023;12:1597.

73. Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, et al. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *Lancet Infect Dis.* 2013;13:155–65.

Figures

(a)



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(c)

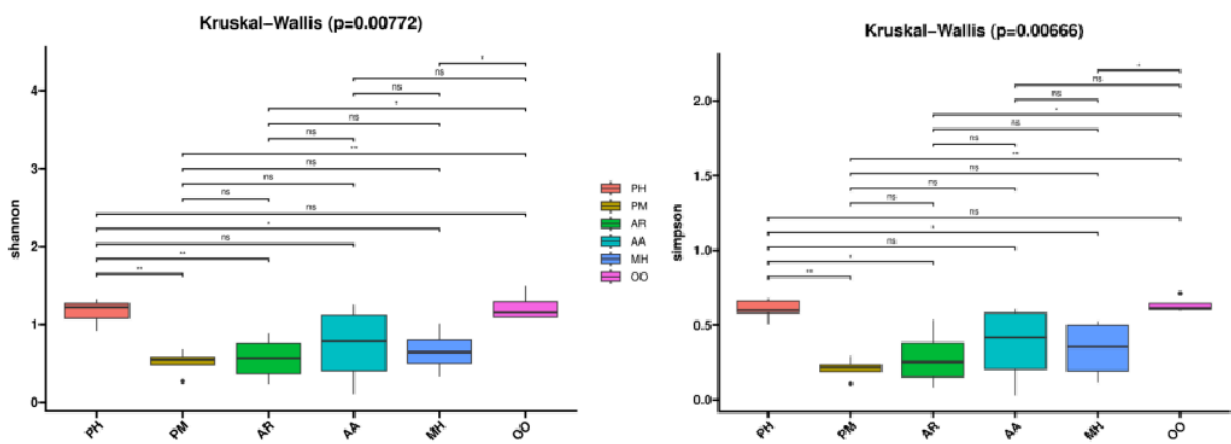
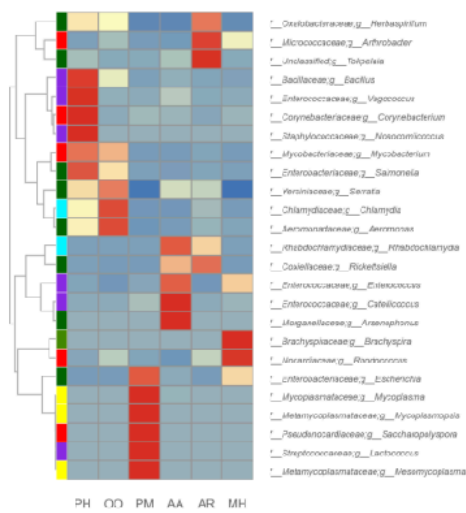


Figure 1

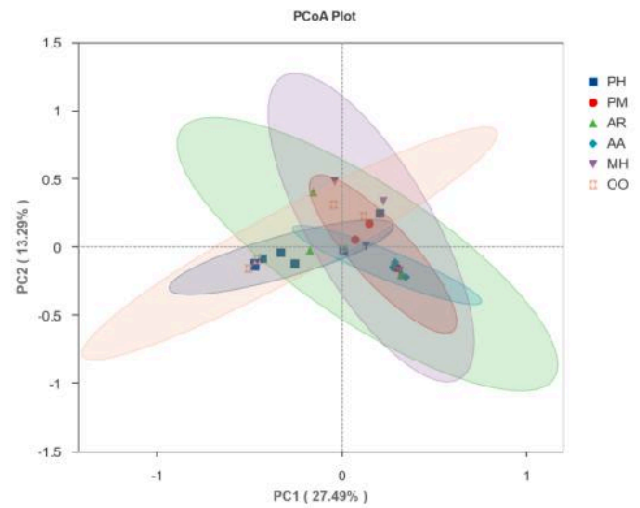
Figure 1

Gut microbial composition and alpha diversity among bird species. (a) Relative abundance of the dominant bacterial phyla. (b) Shannon diversity index. (c) Simpson diversity index.

(a)



(b)



(c)

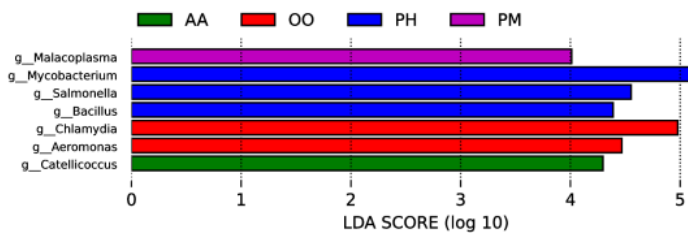


Figure 2

Figure 2

Comparison of the gut bacterial communities among the six bird species. (a) Cluster heatmap of the dominant bacterial genera. (b) PCoA based on the Bray–Curtis distance. (c) LEfSe analysis of differentially enriched bacterial taxa.



Figure 3

Figure 3

KEGG functional profiles of metagenomic unigenes in bird gut microbiomes. The bar plot shows the distribution of genes assigned to KEGG level 2 pathways within major level 1 functional categories.

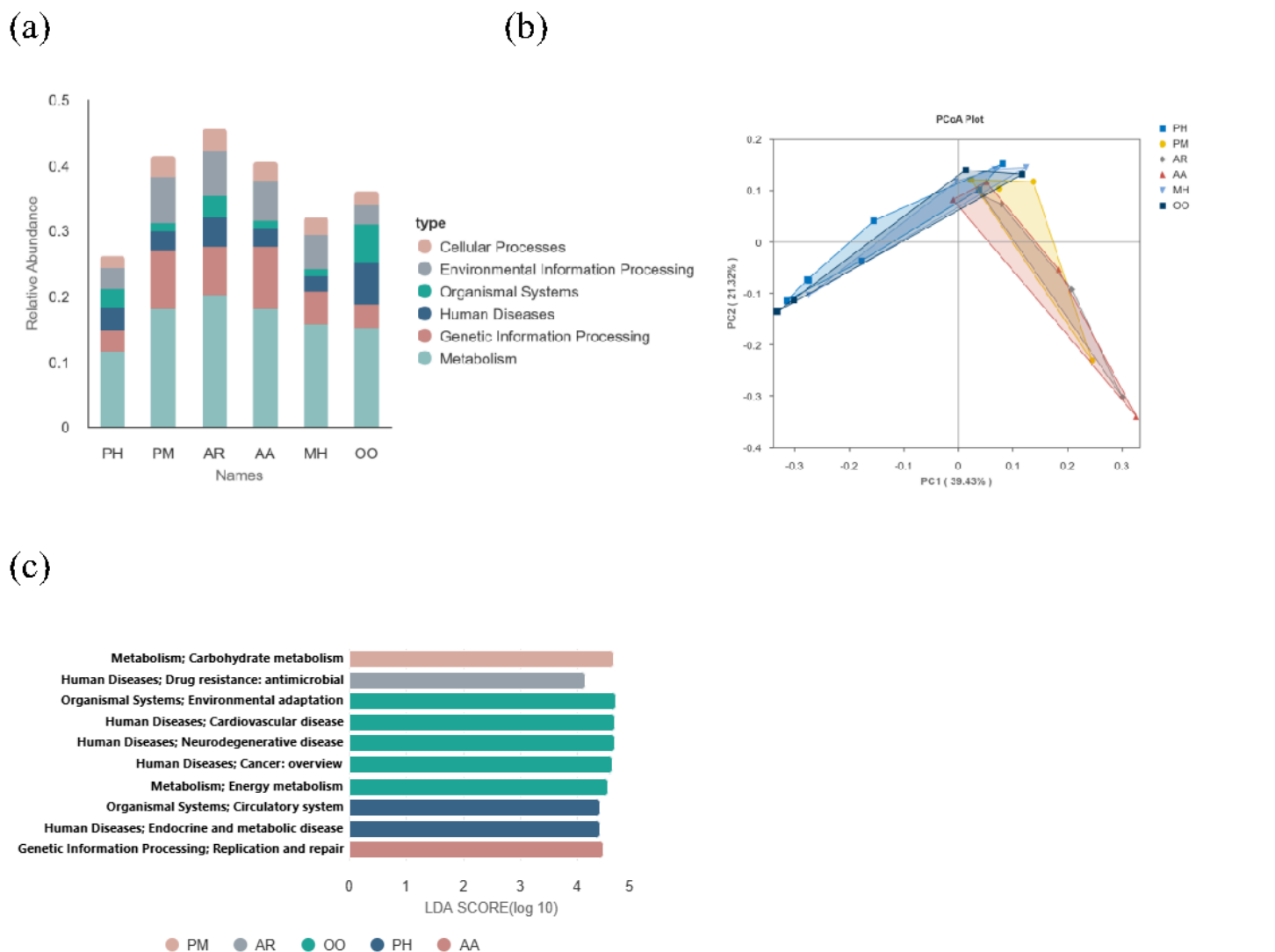
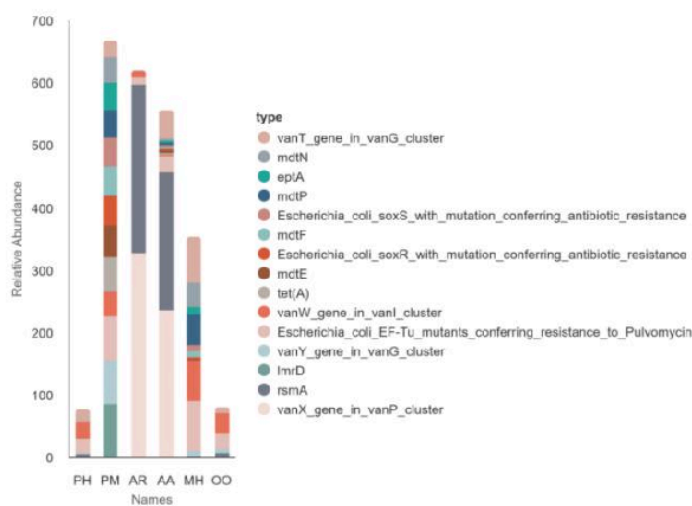


Figure 4

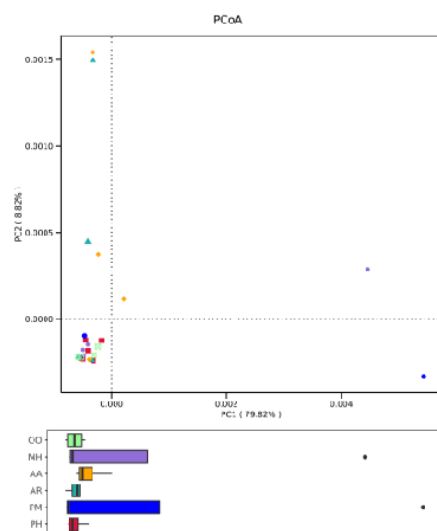
Figure 4

Functional variation in the gut microbiome of wild birds. (a) Relative abundance of major KEGG level 1 functional categories. (b) PCoA based on the Bray–Curtis distance of KEGG functional profiles. (c) LEfSe analysis of significantly enriched KEGG pathways among the bird groups.

(a)



(b)



(c)

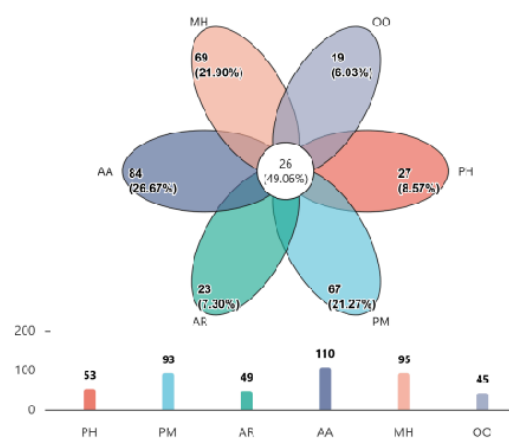


Figure 5

Figure 5

Comparison of the resistome composition among wild bird species. (a) Relative abundance of dominant antibiotic resistance genes (ARGs). (b) Principal coordinate analysis (PCoA) based on the Bray–Curtis distance of the resistome profiles. (c) Shared and unique ARGs among bird species.

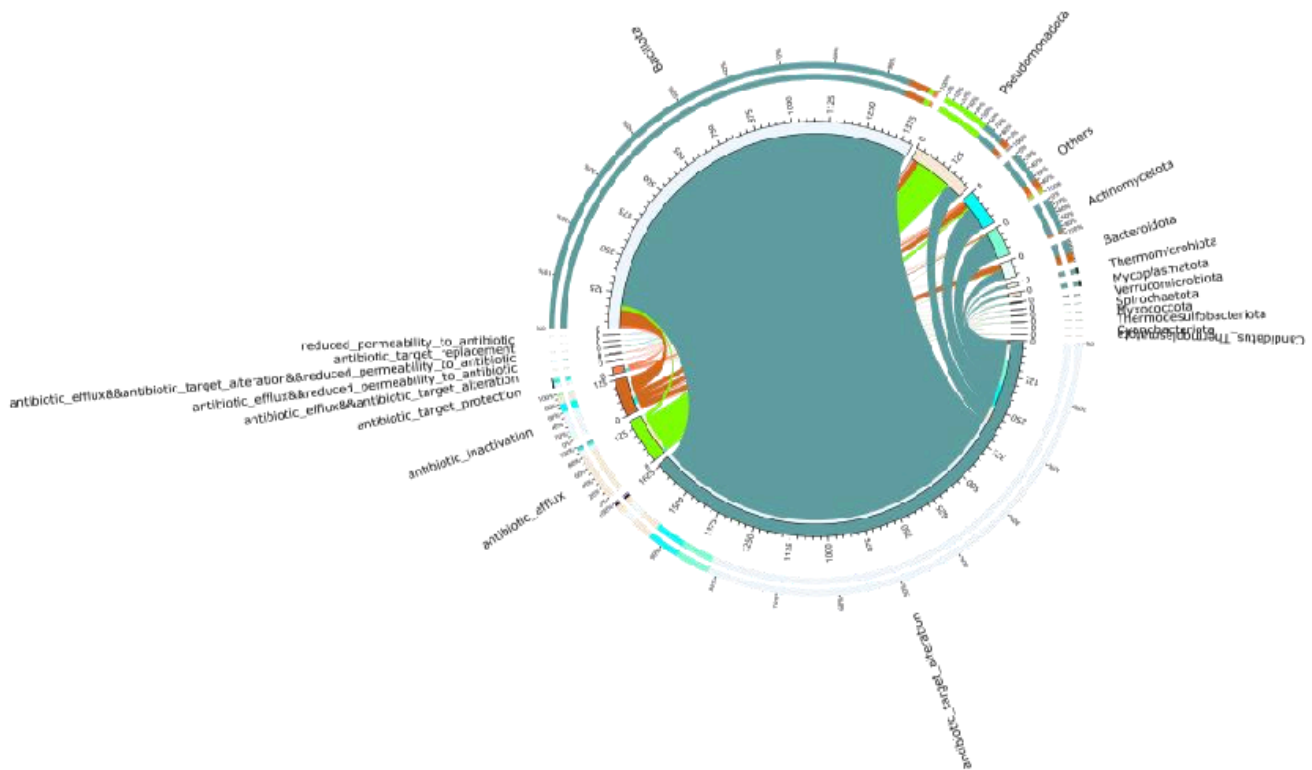


Figure 6

Figure 6

Circos plot showing the associations between bacterial taxa and antibiotic resistance mechanisms in wild bird gut microbiomes.

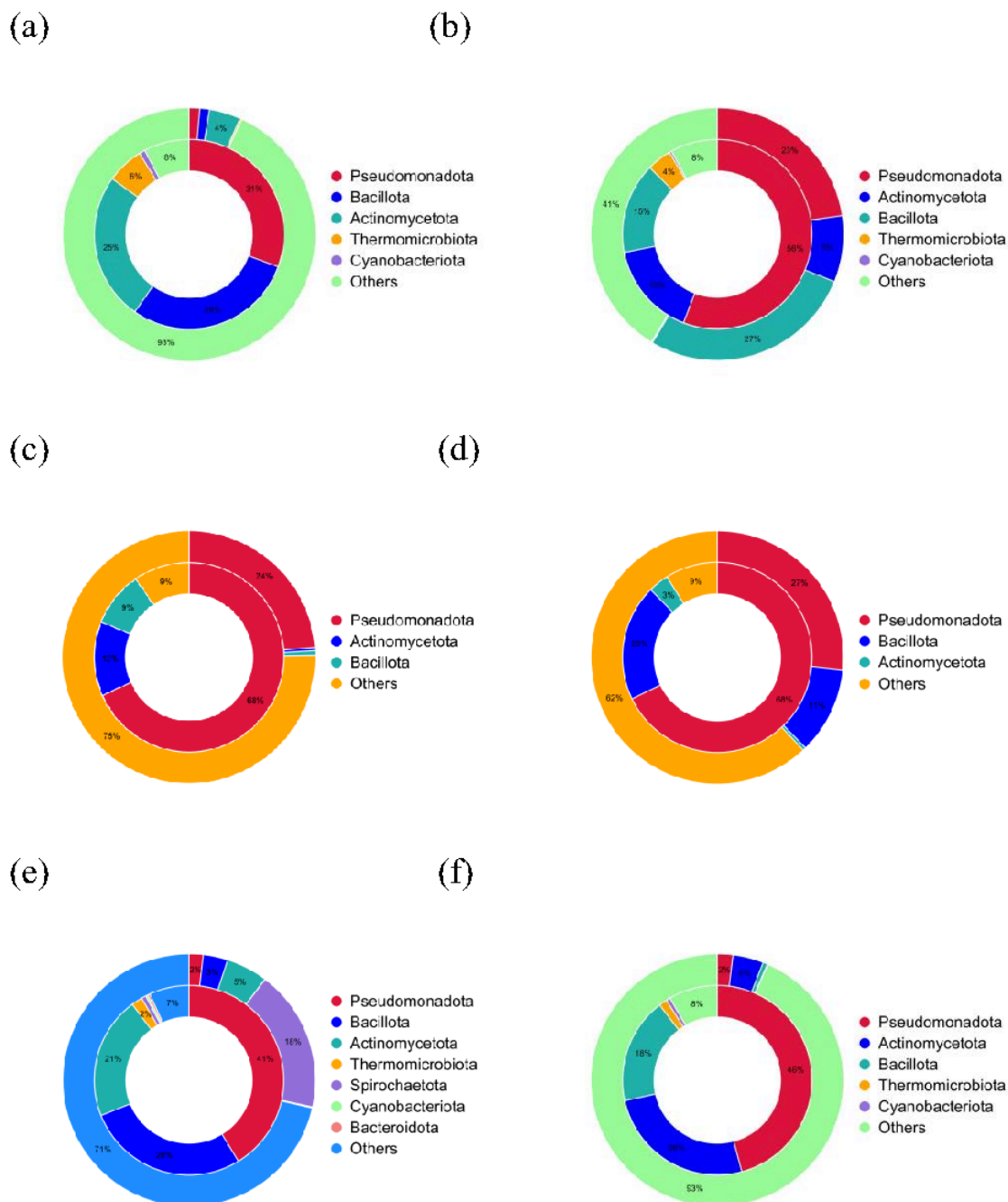


Figure 7

Figure 7

Circos plots representing the associations between microbial phyla and antibiotic resistance genes in six wild bird groups. (a) PH, (b) PM, (c) AR, (d) AA, (e) MH, and (f) OO. The inner ring shows the distribution of antibiotic resistance genes within different microbial phyla, whereas the outer ring shows the relative abundance of the microbial phyla in each bird group.

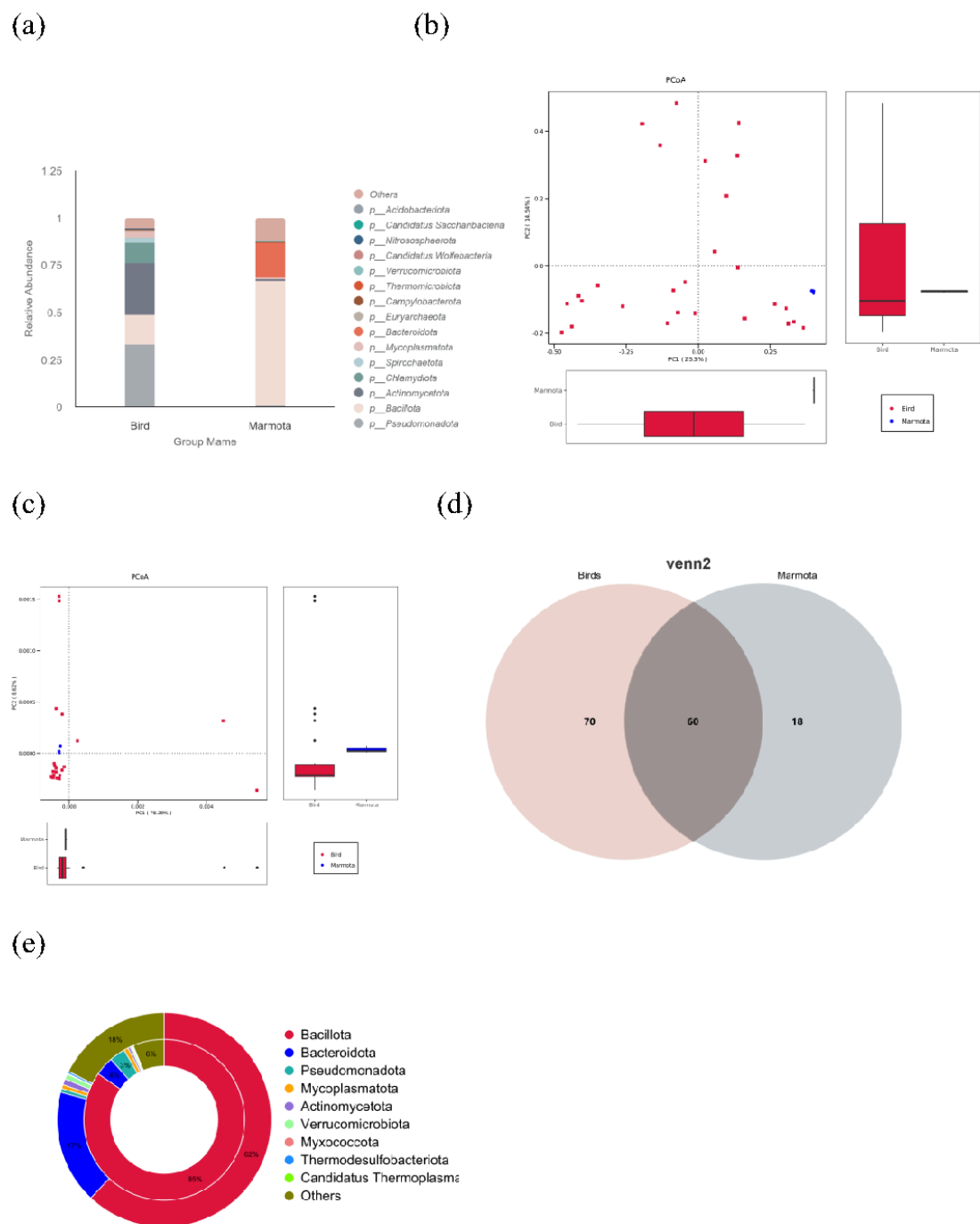


Figure 8

Figure 8

Differences in the microbiome and resistome between birds and *Marmota himalayana*. (a) Bacterial phylum composition. (b) Microbiome PCoA. (c) Resistome PCoA. (d) Shared and unique ARGs. (e) Microbial origins of the ARGs in *Marmota himalayana*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.zip](#)