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Diversity and Regional Variation of the Chicken Microbiome in Poultry in Kazakhstan

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ABSTRACT

The poultry industry plays a crucial role in global food security because the chicken microbiome significantly influences bird health, productivity, and disease transmission. However, regional differences in the microbiome composition remain underexplored, particularly in Kazakhstan. This study used high-throughput sequencing to analyze the taxonomic diversity of the chicken microbiome across multiple regions of Kazakhstan, identifying key microbial taxa and potential pathogens. A total of 108 tracheal and cloacal swabs were collected from private farms, and metagenomic sequencing was conducted using the Ion Torrent PGM platform. Taxonomic classification identified genera including *Chlamydia*, *Pseudomonas*, *Avibacterium*, *Gallibacterium*, and *Aeromonas*, some of which are associated with poultry diseases and zoonotic risks. Alpha and beta diversity analyses showed microbiome variation between regions, indicating that environmental and management factors shape the microbiota composition. These findings underscore the importance of metagenomic surveillance for poultry health management, early pathogen detection, and the development of targeted intervention strategies. Understanding the regional microbiome dynamics can contribute to sustainable poultry farming practices, reduce antibiotic dependency, and enhance food safety in Kazakhstan's rapidly expanding poultry sector.

Keywords: Metagenome; NGS; Microbiota; Pathogen; Chicken; Poultry.

INTRODUCTION

Chickens are the most important source of animal protein worldwide and are by far the most popular meat, accounting for approximately 40% of the world's meat production (de Mesquita Souza Saraiva et al. 2022; Mendybayeva et al. 2023; Abreu et al. 2023). The respiratory and gastrointestinal tracts of chickens host diverse microbiomes that play important roles in digestion, nutrient absorption, immune system development, and pathogen elimination (Deng et al. 2021; Fathima et al. 2022). These symbiotic interactions between bacteria and chickens are important for poultry health and productivity and affect feed efficiency and growth performance (Shang et al. 2018; Dittoe et al. 2022). A well-balanced microbiome contributes to disease resistance in chickens; conversely, disruptions in the microbiota can negatively affect bird health (Stamilla et al. 2021). Moreover, chickens can carry pathogens that pose a risk to poultry farms. For example, *Salmonella* and *Campylobacter* are significant causes of diseases in humans through the ingestion of poultry or industrial poultry (Rukambile et al. 2021; Bindari and Gerber 2022; Mendybayeva et al. 2023). Therefore, public health protection depends on strategies to manage the chicken microbiome to reduce the burden of zoonotic pathogens (Mekonnen et al. 2024).

In addition to pathogens, the chicken microbiome is also closely linked to antibiotic resistance (Xiao et al. 2023; Liang et al. 2023). For decades, poultry farmers have used antibiotics to stimulate growth and prevent diseases in flocks. This approach improved production but put selective pressure on gut bacteria, leading to the emergence of antibiotic-resistant strains in the chicken microbiome (Abreu et al. 2023). As a result, the intestinal microbiota of chickens serves as a reservoir for antibiotic resistance genes ("resistome") (Xu et al. 2022; Vieira et al. 2023). This leads to the spread of antibiotic resistance from commensal bacteria in poultry to pathogenic bacteria that

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cause disease in humans. Thus, antimicrobial resistance in poultry has become a major public health problem. Studies on the gut microbiota of chickens have been limited to culture-based methods, which can identify only a small proportion of bacteria. The advent of high-throughput sequencing has enabled the identification of bacterial and viral profiles in poultry microbiomes (Shang et al. 2018; Segura-Wang et al. 2021). In one study, scientists reconstructed more than 5,000 microbial genomes from chicken fecal samples and identified more than 800 bacterial species (Gilroy et al. 2021).

Despite advances in the study of the chicken microbiome, significant knowledge gaps remain. Most studies have been conducted in regions such as North America, Europe, and East Asia, whereas other areas of the world are poorly represented. Data analysis indicates that only a few metagenomic studies of the chicken microbiome have been published in Kazakhstan. Given that the poultry industry is rapidly developing in Kazakhstan, a lack of knowledge may negatively affect it (Mendybayeva et al. 2023). Factors such as management practices, feed, regional location, climate, and common chicken breeds in Kazakhstan can uniquely shape the gut microbiome (Shang et al. 2018). In addition, the types of antibiotics used may differ between regions of Kazakhstan and other countries. In turn, this can lead to distinct taxonomic and resistance gene profiles in the microbiome. Due to limited information on the chicken microbiome in Kazakhstan, it is difficult to assess the risks posed by pathogens or determine the extent of antibiotic resistance on poultry farms (Korotetskiy et al. 2025).

Kazakhstan has begun to modernize its poultry industry. Government programs (e.g., the Poultry Development Program for 2018-2027) focus on reducing antibiotic use and improving biosecurity and food safety. There is growing interest in the development and implementation of probiotic feed additives and other alternatives to antibiotics in the poultry industry (Temirbekova et al. 2024). However, the success of such efforts depends on understanding the current state of the chicken microbiome in poultry farms (Yang et al. 2022). This underscores the need for regional studies to establish benchmarks for healthy flocks and identify marker pathogens or resistance genes.

This study aimed to assess the regional taxonomic profile of the chicken microbiome across Kazakhstan to identify potential pathogens and create an evidence base for procedures aimed at improving poultry health, reducing the spread of pathogens, and strengthening food safety.

MATERIALS AND METHODS

Collection samples

A total of 108 swab specimens from the trachea and cloaca were collected from chickens raised on private farms across various Kazakh regions, including Almaty, Karaganda, Petropavlovsk, Saryagash, Shymkent, and Turkestan. At each sampling site, representing either a single poultry house or an area where birds gathered, a minimum of five samples were obtained (Table 1). Swabs were taken using sterile cotton applicators, which were immediately immersed in tubes containing 2mL of transport medium (Viral Specimen Collection Tube, China). Collection, storage, and transposition of the received samples were carried out in accordance with the sanitary rules at 4°C. To ensure proper preservation of the samples, they were promptly placed in a sealed icebox after collection and thereafter maintained in a biomedical freezer at -80°C upon their arrival at the laboratory.

Sequencing

The collected samples were grouped by geographic origin, yielding six combined pools per sampling location. Extraction of bacterial DNA was performed using the PureLink Genomic DNA Mini Kit (Invitrogen, USA). Viral RNA and DNA were extracted using the Viral RNA/DNA Mini Kit (Invitrogen, USA) according to the manufacturer's guidelines. Each pooled sample, containing both DNA and viral RNA, underwent reverse transcription (RT) and subsequent double-stranded DNA synthesis using the QIASeq Stranded RNA Library Kit (Qiagen, Hilden, Germany) and random hexamer primers at 100pmol. The DNA obtained was quantitatively and qualitatively assessed using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) at 260nm and 280nm.

The Ion Torrent Personal Genome Machine (PGM) platform was subsequently used for sequencing. Libraries were prepared with the Ion Xpress Plus Fragment Library Kit (Life Technologies, USA). The integrity and fragmentation quality of these libraries were evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). Multiplex sequencing involved the incorporation of barcodes into DNA reads by the Xpress Barcode Adapters Kit (Life Technologies, USA). Sequencing runs were executed on a 318 chip with the Ion PGM Hi-Q View Sequencing Kit.

Post-sequencing data analysis was conducted using various software packages under default conditions, as detailed subsequently. The raw metagenomic data generated in this study are available in the NCBI database under BioProject PRJNA1141889 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1141889).

Taxonomic classification

Taxonomic analysis was carried out using Kaiju (Menzel et al. 2016), integrated into KBase (Arkin et al. 2018), set to operate in Greedy mode. The parameters used were: (1) a minimum match length of 15 nucleotides, (2) a Greedy minimum Bitscore threshold of 65, (3) a maximum allowance of three mismatches, and (4) a Greedy maximum e-value threshold set at 0.01 to exclude low-complexity sequences. Taxonomic identification was conducted against the NCBI BLAST nr reference database, excluding eukaryotic sequences. The resulting classifications obtained with Kaiju were visualized as stacked bar plots.

Alpha diversity was estimated using the Simpson, Shannon, and Chao1 indices in the “vegan” R package (Oksanen et al. 2025). To evaluate statistical significance in alpha diversity across the studied regions, a nonparametric Kruskal-Wallis test was applied.

Beta diversity among samples was analyzed using principal coordinate analysis (PCoA) constructed from Bray-Curtis dissimilarity matrices. Data were preprocessed to eliminate scale differences between variables. The distance matrix was calculated using the “vegdist” function from the “vegan” package in R. The statistical

computations, comparative analysis of the metagenomic data, and graphical visualization were conducted in R version 4.4.2 (R 2025), utilizing the packages "vegan" and "ggplot2" (Wickham 2016).

To assess the statistical significance of the influence of the factor "Region" on the structure of taxonomic composition of communities, PERMANOVA analysis was performed using the adonis2 function of the vegan package in R (2025). Testing was performed on the Bray-Curtis matrix of pairwise distances.

RESULTS

The analysis included 108 tracheal and cloacal swabs from six regions of Kazakhstan (Petropavlovsk, Karaganda, Saryagash, Turkestan, Almaty, and Shymkent). The geographical distribution of the samples is shown in Fig. 1 and Table 1.

Eight pooled samples were included in this analysis. The summary sequencing metrics (number of reads, average length, quality distribution, etc.) are presented in Table 2.

The taxonomic profiles of the samples at the genus level are summarized in Fig. 2. The relative proportions of genera for each sample were presented, allowing a visual comparison of the microbial community composition and variability between samples (Fig. 2).

The bar chart shows the genus composition of the microbiota by region (bird sample pools), classified using Kaiju and NCBI-nr. Viruses were noted separately. The rarefaction curve indicated whether the sequencing depth of the metagenomic sample was sufficient to recover all or most of the species inhabiting the ecological niche. At the saturation point, the highest number of taxa detected corresponded to observed species richness. Fig. 3 shows the rarefaction curves for intestinal microbiota samples from six regions of Kazakhstan.

The species accumulation curves show a transparent gradient in taxa richness between regions; the highest predicted species saturation was observed in Almaty (the curve continues to rise to coarse read depth), whereas for the other pools, the curves quickly plateau at lower numbers of taxa, indicating lower richness and sufficient sequencing depth to estimate them.

To assess bacterial richness and diversity, alpha-diversity indices, such as Simpson, Shannon, and Chao1, were used. These metrics provided a quantitative evaluation of species abundance and evenness in the individual samples (Fig. 4). Using the nonparametric Kruskal-Wallis test, it was found that the distribution of biodiversity was not statistically different between regions (p=0.429).

Table 1: Summary table of farm and bird characteristics in the regions of Kazakhstan

#	Region	N samples	Host age (days)	Host type	Latitude	Longitude	Cross
1	Almaty	18	294	laying hen	43.456	76.813	arbor acres
2	Karaganda	20	332	laying hen	49.859	73.259	n/d[1]
3	Petropavlovsk_1	20	358	laying hen	54.504	69.104	n/d
4	Petropavlovsk_2	20	578	laying hen	54.504	69.104	n/d
5	Shymkent_1	5	623	laying hen	42.341	69.499	nick
6	Shymkent_2	5	197	laying hen	42.341	69.499	nick
7	Turkestan	10	300	laying hen	43 .304	68.268	brown nick
8	Saryagash	10	196	laying hen	41.466	69.181	coral

[1] n/d – not detected.

Table 2: Sample characteristics

Region	Number of reads	Total number of bases	Mean read length	Read length standard deviation	Phred type	Quality score mean	SRA number
Almaty	936587	162123559	173.100	51.647	33	27.38	SRX25510559
Karaganda	168008	22597994	134.506	48.115	33	28.83	SRR32106362
Petropavlovsk_1	362444	51693560	142.625	48.755	33	28.65	SRR32106361
Petropavlovsk_2	300666	43709256	145.375	44.873	33	28.82	SRR32106360
Shymkent_1	126745	17113163	135.020	50.736	33	27.37	SRX25510560
Shymkent_2	71250	9930331	139.373	50.906	33	27.68	SRX25510561
Turkestan	29109	4596283	157.899	48.461	33	27.96	SRX25510562
Saryagash	32912	5146125	156.360	42.399	33	28.23	SRX25510563

Fig. 1: Locations of sample collection spots in six regions of Kazakhstan.

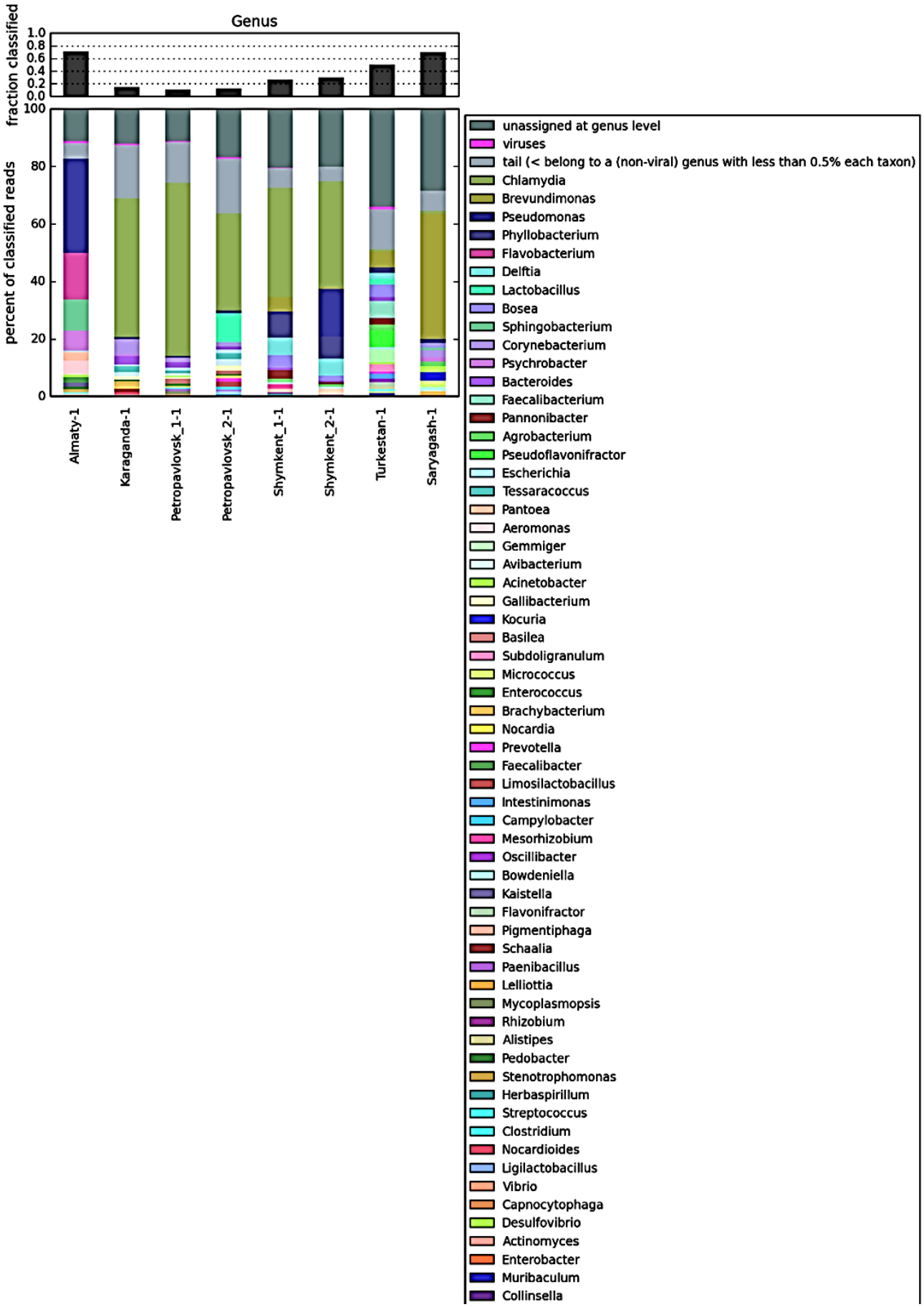

Fig. 2: The relative frequency of the most abundant bacterial genus was identified in the samples collected from different regions of Kazakhstan.

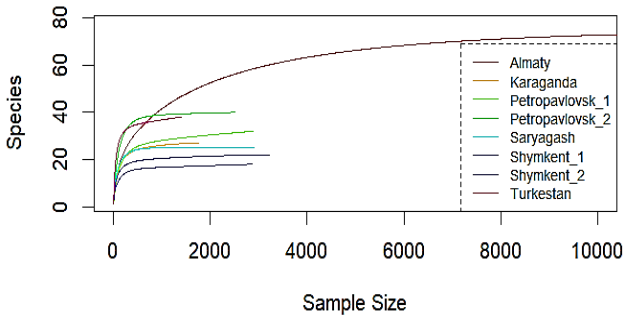

Fig. 3: Rarefaction curve.

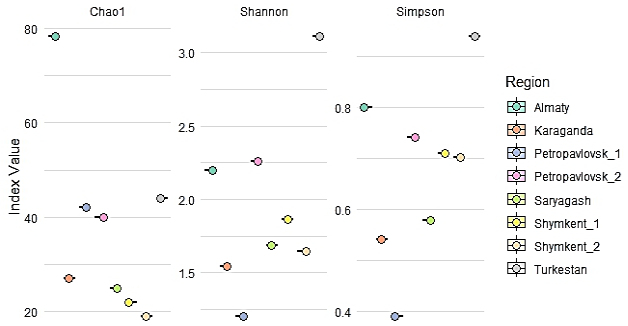

Fig. 4: Alpha diversity of avian gut microbiota taxa by region.

As shown in Fig. 4, Almaty showed the highest richness (Chao1) and diversity/equalization (Shannon, Simpson), while Turkestan showed the lowest values for all three metrics. The medium levels are characteristic of Karaganda and Petropavlovsk_1. Petropavlovsk_2 was at the top of the range (high Shannon/Simpson with moderate Chao1). Samples from Shymkent_1 and Saryagash showed reduced richness and diversity, whereas Shymkent_2 combined low richness (Chao1) with a relatively even distribution of taxa (increased Simpson).

PCoA based on Bray-Curtis distances revealed differences in community structure across regions of Kazakhstan (Fig. 5).

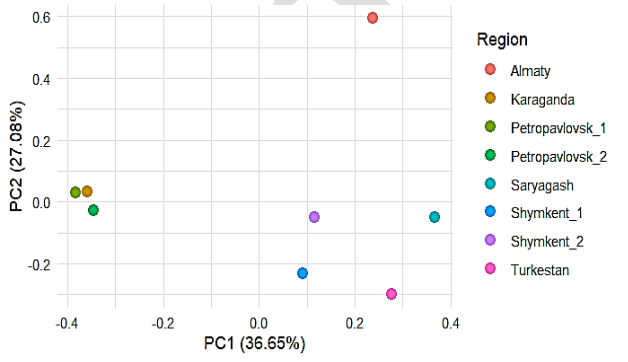

Fig. 5: PCoA analysis of avian gut microbiota taxa by region.

The first two coordinates explained the bulk of the variation (PC1=36.65%, PC2=27.08%). The ordination shows a clear regional structure. Almaty was separated from all other samples. The samples from Karaganda, Petropavlovsk_1, and Petropavlovsk_2 formed a separate group. Turkestan, Shymkent_1, Shymkent_2, and Saryagash were also located in a separate group with moderate mutual separation.

The PERMANOVA analysis showed that the "Region" factor explained 51.37% of the variation in taxon structure ($R^2 = 0.514$), indicating its importance in shaping species composition. However, the lack of statistical significance (P=0.248) may be due to limited data or insufficient power. Nevertheless, the results indicate differences between regions that require further investigation with additional factors and a more detailed analysis.

Subsequently, at the genus level, dominant taxa were identified in each region. The analysis included taxa with relative abundances exceeding 1%.

A study of the intestinal microbiota of birds revealed the presence of various pathogenic microorganisms, among which several taxa associated with the development of serious diseases were distinguished. Among the samples, representatives of the genus *Chlamydia* were detected in all the studied regions: 66.9% in Karaganda; 77.8% in Petropavlovsk_1; 48% in Petropavlovsk_2; 51% in Shymkent_1; 48.2% in Shymkent_2; and 1.4% in Saryagash and Turkestan. The only region in which the percentage of this taxon did not exceed 1% was Almaty.

In addition to *Chlamydia*, other potentially pathogenic taxa, such as *Pseudomonas*, *Avibacterium*, *Gallibacterium*, and *Aeromonas*, have been identified in samples that can cause serious diseases in industrial poultry. In three regions —Petropavlovsk_2, Shymkent_1, and Saryagash —the proportion of Pseudomonas did not exceed 2%, suggesting a normoflora. However, in the Almaty and Shymkent_2 regions, the shares of *Pseudomonas* were 37.2% and 21.8%, respectively. Representatives of the genus *Avibacterium* were found in samples from Karaganda (1.7%), Petropavlovsk_1 (1.2%), and Petropavlovsk_2 (3.2%). *Avibacterium*, a member of the family Pasteurellaceae, includes Avibacterium paragallinarum, the causative agent of infectious rhinitis (infectious coryza) in chickens, making it potentially pathogenic. *Gallibacterium* is another member of the *Pasteurellaceae* family and is associated with reproductive tract infections and septicemia in birds. This taxon was recorded in two regions: Karaganda (1.7%) and Petropavlovsk_2 (2.5%). The taxon *Aeromonas* is a potential pathogen of birds and was detected only in Almaty (5.4%). The intestinal pathogen *Aeromonas hydrophila* can cause enteritis, septicemia, and other infections.

The samples captured commensal bacteria that contribute to the normal functioning of the poultry intestines. The intestinal microflora of birds is a complex and dynamic community of microorganisms that plays a key role in maintaining homeostasis, digestion, and immune defense of the host. Commensal microflora not only contributes to efficient digestion but also participates in the synthesis of bioactive compounds, the regulation of immune response, and defense against pathogenic agents. Karaganda (*Corynebacterium* and *Bacteroides*) is the poorest region for commensal bacteria. The Turkestan region is the richest in commensals (17 taxa).

A large proportion of the identified genera were bacteria, for which there was little information on their affiliation to commensals or pathogens. Some taxa are phytopathogens (*Agrobacterium*, *Mesorhizobium*, *Lelliottia*, and others) and are therefore not representative of the chicken gut microbiota. There is insufficient

information in open sources for the other part of the identified bacteria.

Further studies are needed to fully understand the role of these taxa in the intestines of birds and their impact on their health. Metagenomic analysis of the virome showed that the proportion of identified viral reads was low, representing <0.02% of all data generated. Nevertheless, we analyzed the viral taxa identified in the samples separately. Kaiju tables, focusing on viral genera at the genus level, were generated for each sample, providing qualitative and quantitative insights into viral taxon abundance. *Myoviridae*, *Siphoviridae*, *Podoviridae*, *Autographiviridae*, and others were registered. The viral families *Retroviridae* and *Poxviridae*, which include potentially pathogenic species, were identified; however, their relative abundances remained low (P<0.01).

DISCUSSION

It was found that the chicken microbiota harbors bacterial and viral pathogens with potential adverse effects on poultry and, in some cases, on human health. The regional structure of the community was evident in our data, but the PERMANOVA did not reach statistical significance (R^2 = 0.51; P = 0.248). Consequently, the hypothesis about the influence of the regional factor requires testing on extended samples, and the conclusions about regional differences should be interpreted as tentative.

The high proportion of taxa belonging to the genus *Chlamydia* is consistent with recent reports of a psittacosis outbreak in Europe in 2023-2024, highlighting occupational risks for poultry workers and bird owners. However, given the limitations of taxonomic binning by reads (false-positive attributions with incomplete bases/short reads), species identification (e.g., *C. psittaci*) should be confirmed using targeted tests (PCR analysis) (WHO 2024; Buddle et al. 2024). The approach we use improves sensitivity but does not eliminate misclassification at low coverage. This determines a conservative interpretation and requires confirmatory tests (Menzel et al. 2016).

The genus *Pseudomonas* includes opportunistic strains with distinct resistance profiles. Current reviews and field studies in the poultry industry have documented the co-occurrence of multiple resistance genes and the transfer of ARGs through the production environment. This strengthens the need for local monitoring of resistance phenotypes/genes, especially in farms with water and sanitation stress (Ramatla et al. 2024; Tigabie et al. 2025). Therefore, local monitoring of antibiotic resistance should be conducted regularly, especially on farms with water and sanitation problems.

We identified members of the genus *Avibacterium*, indicating the risk of infectious coryzae in industrial poultry production (Soriano-Vargas 2024). *Gallibacterium anatis* has also been identified. *Gallibacterium* can form biofilms and is often resistant to many antibiotics, making it difficult to treat and increasing the biosecurity requirements. The combination of these infections with other respiratory diseases, such as infectious bronchitis or mycoplasmosis, is hazardous. This increases the severity of clinical manifestations and complicates the diagnosis and treatment. These findings are consistent with those of recent reviews and retrospective studies (Abd El-Ghany et al. 2023; Kursa 2024; El-Gazzar et al. 2025; Nguyen et al. 2025; Nofouzi et al. 2025; Srednik et al. 2025).

The proportion of viral reads in this study was low (<0.02%). This is the result of sample preparation for sequencing. Most of the identified viral taxa belong to normal gut flora (Aruwa et al. 2021; Chrzastek et al. 2021). However, nucleotide sequences corresponding to the viral genera *Retroviridae* and *Poxviridae* were also detected, which may serve as markers of farm welfare. This supports the implementation of panels to expand targeted viral screening, making their detection an important indicator of farm epizootic welfare (Wang et al. 2024).

Collectively, these findings support expanding routine surveillance panels beyond the currently approved targets to include *Chlamydia*, *Pseudomonas*, *Avibacterium*, *Gallibacterium* and marker viral taxa. In the poultry sector in Kazakhstan, risk-based monitoring, enhanced biosecurity, optimized vaccination strategies against respiratory pathogens, and management of co-infections can reduce disease pressure and reliance on broad-spectrum antibiotics. Targeted pathogen detection and early intervention can improve flock health, safeguard workers, and support national antibiotic-reduction initiatives. In the poultry sector of Kazakhstan, risk-based monitoring, enhanced biosecurity, optimized vaccination strategies against respiratory pathogens, and management of co-infections can reduce disease pressure and reliance on broad-spectrum antibiotics. Targeted pathogen detection and early intervention can improve flock health, safeguard workers, and support national antibiotic reduction initiatives.

Conclusion

Metagenomic analysis of the chicken microbiota across diverse regions of Kazakhstan revealed a complex microbial community with both core components and region-specific patterns. The detection of hidden pathogens highlights the value of metagenomic surveillance for early pathogen identification and comprehensive flock health assessments. The obtained microbiota profiles should serve as the basis for industry-specific farming rules and regulatory measures. First, at the national level, it is advisable to establish metagenomic screening as a regular tool for the veterinary surveillance of poultry farming (One Health approach), with mandatory mapping of regional risks and integration of data into a single system. Second, the list of targets for routine monitoring should be expanded to include the indicator taxa identified in this study, and response thresholds and early warning protocols should be established for farms and health care services. In addition, the results obtained may influence policies aimed at reducing antibiotic dependence. Metagenomic monitoring of dysbiosis and hidden pathogens allows a shift from therapeutic to preventive herd management, thereby reducing the frequency of outbreaks and the need for antibiotic therapies. Finally, for industrial practice, it is recommended that biosafety standards and occupational safety regulations for zoonotic risks be updated. This approach, from pathogen registration to regulated actions, will strengthen biosafety, improve production performance, and reduce antibiotic use in poultry farming.

DECLARATIONS

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Conflict of Interest: The authors declare no conflicts of interest.

Data Availability: The raw metagenomic data generated in this study are available in the NCBI database under BioProject PRJNA1141889 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1141889). Accession numbers of SRA are given in Table 2.

Ethics Statement: The protocol was approved by the Committee o*n* Institutional Animal Care and Use *at* the Scientific and Production Center of Microbiology and Virology LLP, Almaty (ID # 02-09-47 from 27.10.2022).

Author's Contribution: Conceptualization: I.K. Methodology: S.S., T.K., L.I. Software: I.K. Validation: N.Z., N.K., and T.I. Formal analysis: S.S., N.Z., and L.I. Investigation: I.K., S.S. Resources: T.I. and N.K. Data curation: T.K. and N.Z. Writing-original draft preparation: I.K. Writing-review and Editing: N.Z., S.S., T.K., L.I., N.K., and T.I. Visualization: I.K., S.S., T.K. Project administration: I.K. Funding acquisition: I.K. All authors have read and agreed to the published version of the manuscript.

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