BMC Microbiology

RESEARCH

Open Access

Metagenomic insights into microbial diversity and potential pathogenic transmission in poultry farm environments of Bangladesh

Afroja Yasmin[1,2†], M. Shaminur Rahman[3†], S. M. Kador[3], Md. Mustak Ahmed[3], Md. Eashanul Karim Moon[3], Humaira Akhter[1], Munawar Sultana[1*] and Anowara Begum[1*]

Abstract

The microbiome plays a critical role in poultry health and productivity, influencing growth, immunity, and the overall farm ecosystem. This study investigated microbial diversity, antibiotic resistance pathways, and functional potential across various components of poultry ecosystems—cloacal swabs, droppings, feed, hand swabs, soil, and water—in different districts of Bangladesh. Using 16S rRNA gene amplicon sequencing, we identified 2,745 Operational Taxonomic Units (OTUs) and analyzed microbial richness, community structure, and functional pathways. Alpha diversity metrics revealed that droppings exhibited the highest microbial richness (726 OTUs in Noakhali), while feed samples showed the lowest diversity (211 OTUs). Beta diversity analysis indicated significant differences in microbial composition across sample sources, with PERMANOVA confirming that sample origin accounted for 51.45% of the variability ($p < 0.001$). Proteobacteria dominated the microbial communities (48.36%), followed by Firmicutes (19.83%) and Cyanobacteria (12.02%). Key genera of concern, such as *Enterobacter* (26.62% in hand swabs), *Acinetobacter* (30.87% in cloacal swabs), and *Shigella* (22.89% in cloacal swabs), were identified, highlighting potential contamination and zoonotic risks. Conversely, beneficial genera like *Lactobacillus* (36.89% in feed) and *Enterococcus* (10.78% in droppings) were prevalent, suggesting roles in gut health and nutrient cycling. Functional pathway analysis (KEGG) revealed that carbohydrate and amino acid metabolism were highly active in droppings and feed, reflecting nutrient utilization. Antimicrobial resistance (AMR) pathways, such as 23S rRNA-methyltransferase and multidrug efflux pumps, were widespread, with pathogenic genera (*Enterobacter*, *Acinetobacter*, *Shigella*, *Pseudomonas*) showing strong positive correlations with AMR pathways. These findings underscore the influence of environmental factors on microbial diversity and functional potential in poultry farming. The study highlights the need for improved management practices and biosecurity measures to mitigate risks associated with microbial pathogens and antimicrobial resistance, ultimately supporting healthier and more sustainable poultry production in Bangladesh.

Keywords Microbiome, Poultry health, Amplicon sequencing, Microbial diversity, Antimicrobial resistance, Bangladesh

†Afroja Yasmin and M. Shaminur Rahman contributed equally to this work.

*Correspondence:
Munawar Sultana
munawar@du.ac.bd
Anowara Begum
anowara@du.ac.bd
Full list of author information is available at the end of the article

Introduction

Microbial diversity in agricultural environments, particularly in poultry farms, plays a critical role in influencing animal health, productivity, and food safety [1]. Poultry farms are hotspots for microbial interactions, where complex communities of bacteria, fungi, and viruses interact in ways that can either promote health or facilitate the spread of pathogens [2, 3]. These microbial ecosystems are shaped by factors such as feed quality, water sources, hygiene practices, and environmental conditions, all of which influence nutrient cycling, disease dynamics, and the transmission of pathogens [4, 5]. Among the pathogens of concern, *Salmonella* spp., *Campylobacter jejuni*, and *Escherichia coli* are particularly significant due to their ability to cause disease in both poultry and humans, posing risks to food safety and public health [6].

The transmission of pathogenic microorganisms in poultry farms occurs through multiple routes, including contaminated feed, water, and direct environmental contact [7]. Understanding these pathways is essential for developing effective strategies to monitor and control pathogen spread. For instance, fecal contamination of drinking water sources or fecal–oral transmission among poultry can lead to the proliferation of pathogens, compromising animal health and increasing the risk of zoonotic transmission [8]. Additionally, farm workers can act as vectors, transferring microorganisms between poultry and the environment, further complicating microbial management [9]. In Bangladesh, where poultry farming is a cornerstone of food security and economic development, addressing these microbial risks is critical for improving productivity and ensuring public health [10].

Recent advances in metagenomics, particularly 16S ribosomal RNA (rRNA) gene sequencing, have revolutionized our ability to analyze microbial communities in poultry environments [11]. This culture-independent approach allows for the identification of microbial taxa and the characterization of community dynamics, providing insights into the roles of specific microorganisms in health and disease [12]. However, while 16S rRNA sequencing is a powerful tool for taxonomic identification, it has limitations in predicting functional potential and pathogenicity. For example, the detection of antimicrobial resistance genes (ARGs) or pathogenic taxa using 16S data alone remains putative, requiring complementary approaches such as shotgun metagenomics or functional assays for validation [13].

Despite these limitations, 16S rRNA sequencing has been instrumental in advancing our understanding of poultry microbiomes. Studies have characterized microbial communities in the gastrointestinal tract, respiratory system, and farm environments, revealing the influence of dietary supplements, management practices, and environmental factors on microbial diversity and function [14, 15]. For example, research has shown that feed composition and water quality significantly impact gut microbiota and ARG profiles, highlighting the need for targeted interventions to mitigate microbial risks [16]. In Bangladesh, where farming practices and environmental conditions differ from global trends, understanding the unique characteristics of poultry microbiomes is essential for developing locally relevant strategies to improve productivity and food safety [17].

This study focuses on the microbial diversity and functional potential of poultry farm environments in Bangladesh, with an emphasis on identifying transmission pathways and microbial risks. We hypothesize that microbial communities vary significantly across sample sources (cloacal swabs, droppings, feed, hand swabs, soil, and water) and geographic locations, and that these variations correspond to differences in functional potential, including ARG abundance. By analyzing microbial diversity and composition using 16S rRNA sequencing, we aim to identify potential sources of contamination, assess the effectiveness of current hygiene practices, and inform strategies to mitigate microbial risks. Our findings will contribute to improved farm management, biosecurity, and antimicrobial resistance (AMR) mitigation, ultimately supporting healthier and more sustainable poultry production in Bangladesh.

Materials and methods

Ethics approval and consent to participate

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approvals were obtained from the Institutional Animal Care and Use Committee (IACUC) and the Ethics Review Committee of the Faculty of Biological Science, University of Dhaka (Approval No. 288/Biol. Scs; December 12, 2024) for both animal and human research components. For the animal component, cloacal swabs were collected from live chickens following strict welfare guidelines to minimize stress and discomfort, with all procedures performed by trained personnel using standard biosecurity protocols. For the human component, hand swabs were collected from poultry workers after obtaining written informed consent. Participants were fully informed about the study objectives, procedures, voluntary nature of participation, confidentiality measures, and their right to withdraw without consequences. All samples were handled following established biosafety protocols to ensure participant safety and data protection. The study design and protocols were reviewed and approved by the respective ethics committees prior to implementation. Clinical trial number: not applicable.

Sample collection and processing

The study was conducted across three distinct poultry farms located in the Noakhali, Cumilla, and Gazipur districts of Bangladesh (Fig. 1). With the permission of farm owners from each farm, six types of samples were collected: poultry litter, cloacal swabs, droppings, worker's hand swabs, chicken drinking water, and soil, resulting in a total of 18 samples. General hygienic conditions on the farm were assessed as poor. Biosecurity measures were implemented, though adherence was moderate, reflecting common practices in local Bangladeshi poultry farming. Samples were collected aseptically from three different locations within each farm to ensure representativeness. Poultry droppings (5 gm) were collected from different parts of the cage using sterile cotton buds and immediately transferred into sterile falcon tubes. Cloacal swabs were collected from different healthy layer chickens, vaccinated with Newcastle disease virus about month prior to sample collection. The chickens were at 22 weeks of age with average body weight 2000 gm. Cloacal swabs were obtained by inserting sterile cotton buds into the cloaca of the poultry and taking swab, which were then

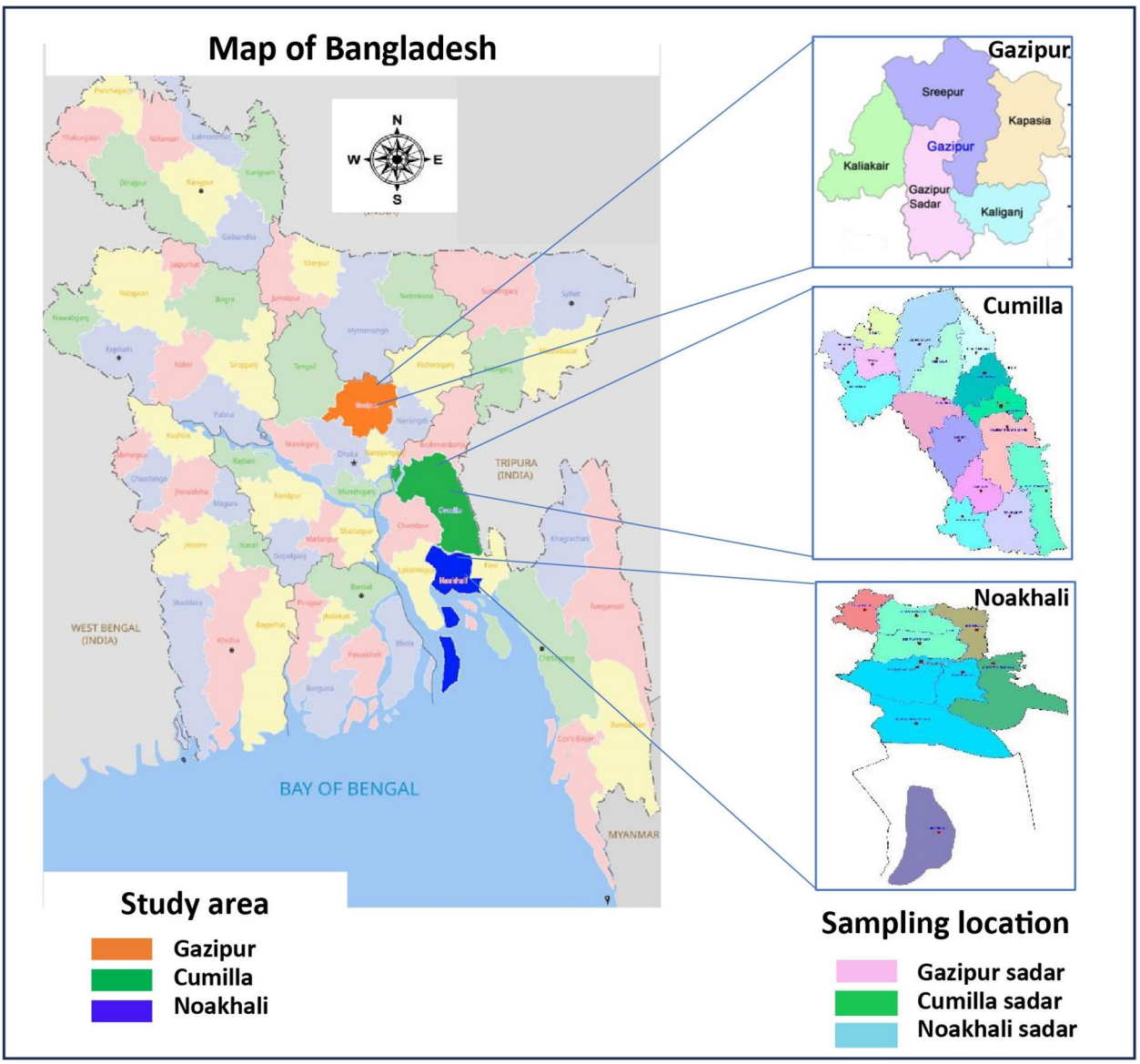

Fig. 1 Map of the study area and sampling locations. Bangladesh Map: https://en.wikipedia.org/wiki/Districts_of_Bangladesh#/media/File:BD_Map_admin.svg. Gazipur district: https://www.scirp.org/journal/paperinformation?paperid=58994. Cumilla district: https://oldweb.lged.gov.bd/DistrictHome.aspx?districtID=10. Noakhali district: https://oldweb.lged.gov.bd/uploadeddocument/DistrictMap/noakhali/noakhali.jpg

placed into falcon tubes containing 5 mL of sterile phosphate-buffered saline (PBS). Worker hand swabs were collected after performing tasks without washing hands; the worker was at bare hand but the samples were collected wearing hand gloves. Like cloacal swab, worker hand swabs were also collected with sterile cotton buds, swabs of the worker's hand were taken then the cotton bud dipped into Falcon tubes containing 5 mL PBS immediately after collection. Poultry feeds (5 gm from each part) were collected in aseptic conditions from different parts and stored in sterile Falcon tubes. Poultry drinking water samples were also collected aseptically from different waterer of the cage. About (500 mL per part) poultry's drinking water were collected using sterile containers. Soil samples were taken from different part. About 5 gm Soil samples were taken from each area, outside of the poultry farms which is adjacent to the poultry farm wall, and stored in sterile tubes. Throughout the sample collection, sterile gloves were worn to maintain aseptic conditions. The samples were promptly placed in an insulated icebox for transportation and arrived at the laboratory within 2–5 h. Upon arrival, the samples were processed immediately and stored at − 20 °C to prevent any degradation before DNA extraction.

Total DNA extraction and 16S rRNA sequencing

Two different DNA extraction kits, DNeasy Powersoil Pro Kit (QIAGEN) and DNeasy Blood and Tissue Kit (QIAGEN), and following the manufacturer's instructions, all 18 samples were treated to extract all of the DNA content. To amplify the V3-V4 region of the bacterial 16S rRNA gene, the primer pairs 341 F (5′-CCTAYG GRBGCASCAG- 3′) and 806R (5′-GGACTACNNGGG TATCTAAT- 3′) that included the Illumina overhang adapter sequence (Illumina, Inc., San Diego, CA, USA) were used. All PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2 µM of forward and reverse primers, and 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃ for 30 s and finally 72℃ for 5 min. Equal volume of 1X loading buffer (contained SYB green) was mixed with PCR products and operated electrophoresis on 2% agarose gel for detection. PCR products were mixed in equidensity ratios. The mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina NovaSeq platform and 2× 250 bp paired-end reads were generated.

Taxonomic profiling of the amplicon sequences

The sequences were generated from 18 samples using 2× 250 base pair reads, ensuring thorough coverage of the 16S V3-V4 region for subsequent bioinformatics analysis. FastQC v0.11 [18, 19] was employed to assess the quality of the FASTQ files. Trimmomatic v0.39 [20] was then utilized with specific parameters, including a sliding window size of 30, a minimum read length of 100 base pairs, and a minimum average quality score of 20, aimed at removing low-quality ends and adapter sequences [21]. Following quality control, an average of 97,598 read pairs per 16S sample remained (minimum = 87,813, maximum = 135,350). QIIME 2 v2023.5 [22] served as an integrated pipeline for OTU clustering, taxonomic assignment, and phylogenetic estimation [22], utilizing VSEARCH for read merging, sequence dereplication, de novo clustering at 99% identity, and chimera detection [23]. Taxonomic assignment relied on the Greengenes2 database (v2022.10) with 99% OTU clustering [23], trained using 16S sequencing primer pairs and a naive Bayes classifier [24]. Classify-sklearn algorithms were applied to classify the assigned OTUs within the samples [25].

Statistical analysis

The downstream analysis encompassed alpha and beta diversity evaluation, microbial composition profiling, and statistical comparisons using the "phyloseq" package in R software (version 4.3) [26]. OTU counts were normalized through total some scalling (TSS) model. Alpha diversity metrics including observed richness, Chao1, Shannon, Simpson, InvSimpson, and Fisher alpha indices were computed and graphically represented using R packages "vegan" [27], "ggpubr" [28], and "ggplot2" [29]. Differences in microbial abundance and diversity between two locations were assessed using the Wilcoxon rank sum test, facilitated by the "microbiomeutilities" package [30]. Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities was employed for beta diversity measurement. Statistical significance of sample dissimilarities was determined using permutational multivariate analysis of variance (PERMANOVA) with permutations.

KEGG functional genomics analysis

Picrust2 (https://github.com/picrust/picrust2), a bioinformatics tool renowned for its utilization in microbiome research, was employed to ascertain the functional genomic potential inherent within microbial communities derived from studied samples. Following data processing via Picrust2 [31] KO (KEGG Orthology) numbers were allocated to delineate the metabolic

pathways inherent within the microbial communities. Tools like PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) enable the prediction of functional profiles from 16S data, offering a cost-effective alternative to whole-genome sequencing for investigating AMR pathway patterns [31, 32]. ggpicrust2 R package [33] was used to pathway annotation process from their KO numbers. Heatmap was visualized by using Pheatmap (https://cran.r-project.org/web/packages/pheatmap/pheatmap.pdf) R package and ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) was used to plot the bar diagrams. Antimicrobial resistance mechanisms, including aminoglycoside, beta-lactamase, carbapenemase, cephalosporinase, macrolide, tetracycline, fluoroquinolone, sulfonamide, glycopeptide, oxazolidinone, rifamycin, polymyxin, quinolone, streptogramin, and several resistance genes such as bla, mecA, vanA, erm, tet, aad, aac, ant, aph, streptomycin, kanamycin, tobramycin, amikacin, neomycin, gentamicin, chloramphenicol, trimethoprim, sulfamethoxazole, colistin, daptomycin, linezolid, vancomycin, clindamycin, metronidazole, and blaKPC, blaNDM, blaOXA, mcr, and MRSA were extracted from KEGG data using in-house Python code. The extracted antimicrobial resistance genes (ARGs) were selected based on their clinical and environmental significance, as well as their representation in the KEGG database.

Results

The microbiome significantly influences the health and productivity of poultry, impacting growth, immunity, and overall farm ecosystems. This study investigates the microbial diversity associated with chicken farms across various districts of Bangladesh, analyzing samples from cloacal swabs, droppings, feed, hand swabs, soil, and water. Utilizing amplicon sequencing (16S rRNA), we assessed microbial richness through operational taxonomic units (OTUs) and examined variations in phyla and genera along with their functional properties. Table 1 summarizes the sample collection data, including sequencing depth, number of OTUs, total phyla count, and total genus count. Our findings aim to enhance the understanding of microbial ecology in poultry farming, providing insights for improved management practices and biosecurity measures.

Microbial diversity measure

The analysis of alpha diversity metrics revealed significant variations in microbial communities across different sample sources and locations. Notably, the Observed OTUs indicated that Droppings (D1, D2, D3) harbored the highest diversity, with D3 from Noakhali exhibiting the most considerable richness at 726 OTUs. In contrast, feed samples (F1, F2, F3) displayed lower richness, particularly F1, which recorded only 211 observed OTUs. The Shannon index, which accounts for both richness

Table 1 The table provides information on sample collection sources from chicken farms in different districts of Bangladesh

Sample ID	Sources	Location	Sequencing Depth (QC passed read pairs)	Number of OTU	Total Phyla Count	Total Genus Count
CS1	Cloacal Swab	Cumilla	88,986	268	14	119
CS2	Cloacal Swab	Gazipur	89,801	160	8	75
CS3	Cloacal Swab	Noakhali	88,469	173	7	73
D1	Dropping	Cumilla	94,312	394	20	182
D2	Dropping	Gazipur	89,624	327	13	148
D3	Dropping	Noakhali	129,783	726	44	249
F1	Feed	Cumilla	89,281	211	9	94
F2	Feed	Gazipur	95,000	414	13	176
F3	Feed	Noakhali	135,350	1072	48	391
HS1	Hand swab	Cumilla	93,302	566	22	243
HS2	Hand swab	Gazipur	87,845	307	14	141
HS3	Hand swab	Noakhali	87,813	216	15	105
S1	Soil	Cumilla	91,820	1196	45	420
S2	Soil	Gazipur	88,817	968	39	316
S3	Soil	Noakhali	133,202	1473	58	509
W1	Water	Cumilla	91,368	183	10	81
W2	Water	Gazipur	93,790	576	36	194
W3	Water	Noakhali	88,210	356	15	162

and evenness, revealed that Soil samples consistently maintained high diversity across locations, with S3 from Noakhali reaching a Shannon value of 5.5329, indicating a well-balanced community. In comparison, Cloacal Swab samples from Cumilla and Gazipur showed lower diversity, with CS1 recording a Shannon index of 2.0722. Additionally, the Simpson and InvSimpson indices further confirmed the high diversity in Soil samples, while the Fisher index indicated that Droppings had a greater abundance of rare species compared to other sources (Fig. 2; Supplementary Data 1). These findings suggest that environmental factors associated with sample sources significantly influence microbial community composition and diversity, highlighting the ecological importance of these habitats in shaping microbial landscapes.

The analysis of beta diversity among microbial communities revealed significant differences across sample sources using the Bray–Curtis dissimilarity metric. The PERMANOVA test indicated that sample sources explained a substantial portion of the variability in microbial composition ($R^2 = 0.5145$; $F = 2.5436$; $p < 0.001$). Specifically, the model accounted for 51.45% of the total variation in community structure among the samples, with sources being a statistically significant factor influencing microbial diversity (Fig. 3). This finding highlights the distinct microbial community profiles associated with different sources, suggesting that environmental factors linked to each source contribute to the observed variations in microbial composition. The results underscore the importance of considering sample sources in studies of microbial ecology, as they can significantly shape community dynamics and functionality.

Microbial community structure

A total of 2,745 Operational Taxonomic Units (OTUs) were detected by analyzing the samples using 16S rRNA gene amplicon sequencing with the V3-V4 amplicon region. The data revealed that the vast majority of the microbial community consists of Bacteria (98.08%), with a small proportion of Archaea (1.88%), while unassigned sequences made up only 0.04% of the total samples analyzed. The research also revealed the presence of 63 bacterial phyla and 3 archaeal phyla (*Parvarchaeota, Crenarchaeota, and Euryarchaeota*) across all the samples. The most predominant phylum was Proteobacteria,

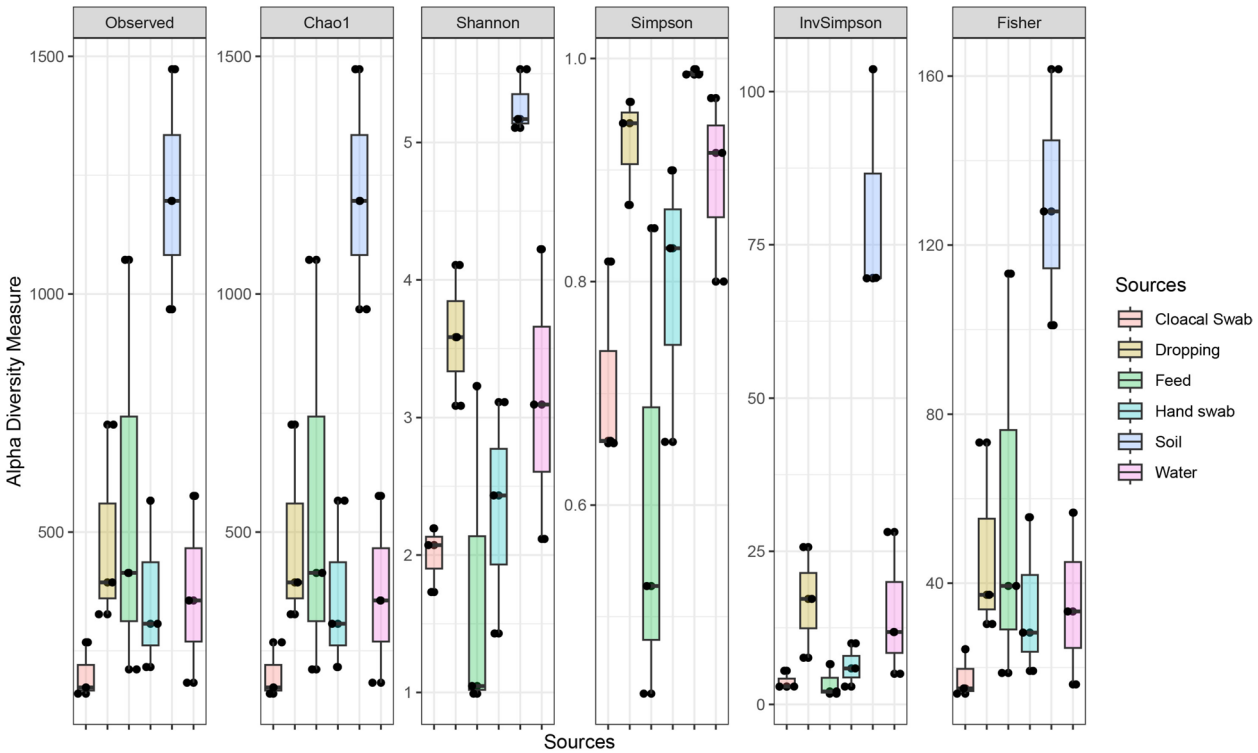

Fig. 2 Alpha diversity metrics of microbial communities across various sample sources and locations. The bar chart illustrates the richness (Observed) and diversity (Shannon, Chao1, Simpson, InvSimpson, and Fisher indices) of microbial communities sampled from different sources, including Cloacal Swabs, Droppings, Feed, Hand Swabs, Soil, and Water, across three locations: Cumilla, Gazipur, and Noakhali. Each bar represents the average alpha diversity measure for samples collected from a specific source. The different colors in the legend correspond to sample sources, allowing for easy comparison of microbial diversity across the varied environmental contexts

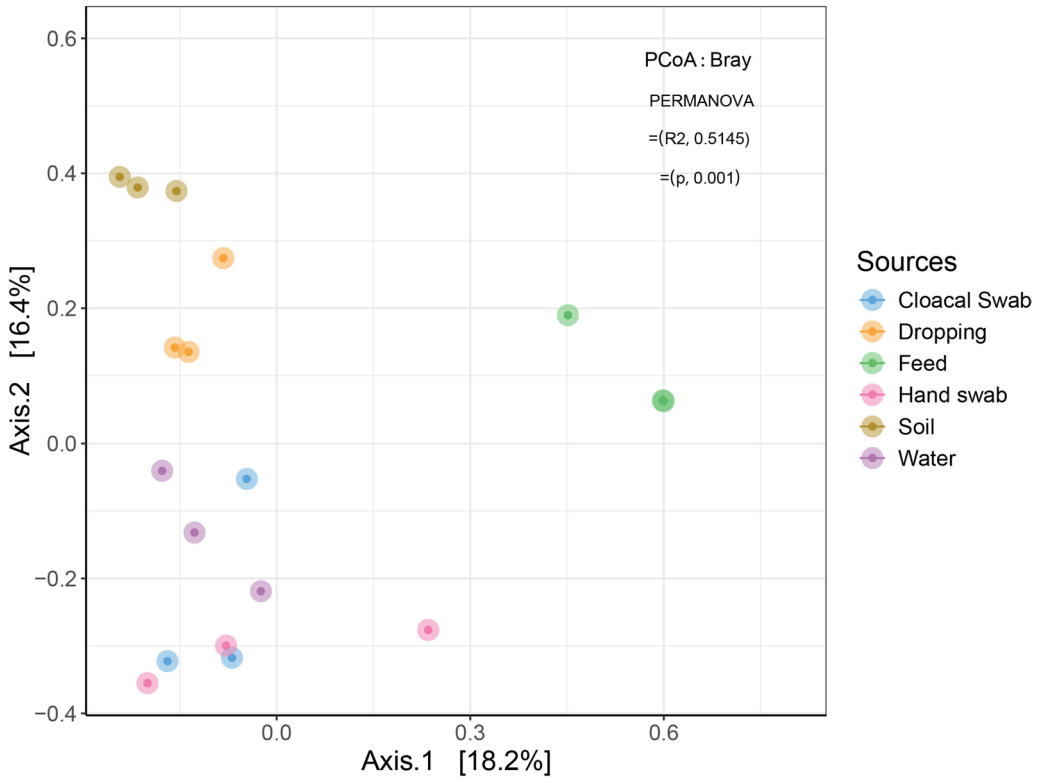

Fig. 3 Principal Coordinates Analysis (PCoA) of microbial communities based on Bray–Curtis dissimilarity, colored by sample sources. The plot displays the distribution of samples in the PCoA space, highlighting differences in microbial composition across various sources. Each point represents a sample. The results of the PERMANOVA analysis are annotated, indicating a significant explanation of variance by sources ($R^2 = 0.5145$; $p = 0.001$). The plot underscores the distinct microbial community structures associated with different sample sources

comprising 48.36% of the total phyla abundance. Firmicutes and Cyanobacteria also represented significant proportions, with percentages of 19.83% and 12.02%, respectively. Additionally, other phyla such as *Bacteroides* (9.52%), *Actinobacteria* (4.07%), and *Acidobacteria* (1.14%), along with *Crenarchaeota*, *Nitrospirae*, *Chloroflexi*, *Parvarchaeota*, *Gemmatimonadetes*, and *Verrucomicrobia*, each representing less than 1%, were identified (Supplementary Data 1).

In terms of sample sources, the study indicated that Bacteria dominate across all sample sources, with the highest prevalence in Cloacal Swab (99.99%) and Dropping (99.94%), followed closely by Feed (99.97%) and Hand Swab (99.98%). In contrast, Archaea were more prominent in Soil (6.17%) and Water (5.02%), while they were present in much lower proportions in other sample types. Unassigned sequences are minimal across all samples, with the highest occurrence in Water (0.19%) and very low percentages in other sources (Fig. 4 A, B, C, D). The analysis revealed distinct patterns of microbial phyla across different sample sources. Proteobacteria were the most abundant in most samples, with the highest prevalence observed in Hand Swabs (77.75%) and Water (54.07%). Firmicutes were particularly abundant in Dropping samples (68.40%) and were present in moderate amounts in other sources. *Cyanobacteria* exhibited high abundance in Feed samples (52.05%) but were less common in other sources. Archaea-related phyla, such as *Parvarchaeota* and *Crenarchaeota*, were more prominent in Soil and Water samples. Bacteroidetes showed significant presence in Water (19.67%), while other phyla, including *Actinobacteria* and *Acidobacteria,* demonstrated variable abundance across different sources, with elevated levels in Soil (Supplementary Data 1).

The analysis of microbial communities in various sample types revealed significant differences in the abundance of different genera. The genus *Acinetobacter* was detected with the highest relative abundance in cloacal swabs (30.87%), followed by droppings (3.40%) and feed (1.37%). *Shigella* exhibited a prominent presence in cloacal swabs (22.89%), while its abundance in other samples remained low, particularly in feed (0.18%). *Enterobacter* was notably abundant in hand swabs (26.62%), with minimal presence in droppings (0.00%) and feed (0.39%). *Bacteroides* showed a higher abundance in droppings (11.54%), followed by feed (0.71%) and soil (2.77%). *Pseudomonas*

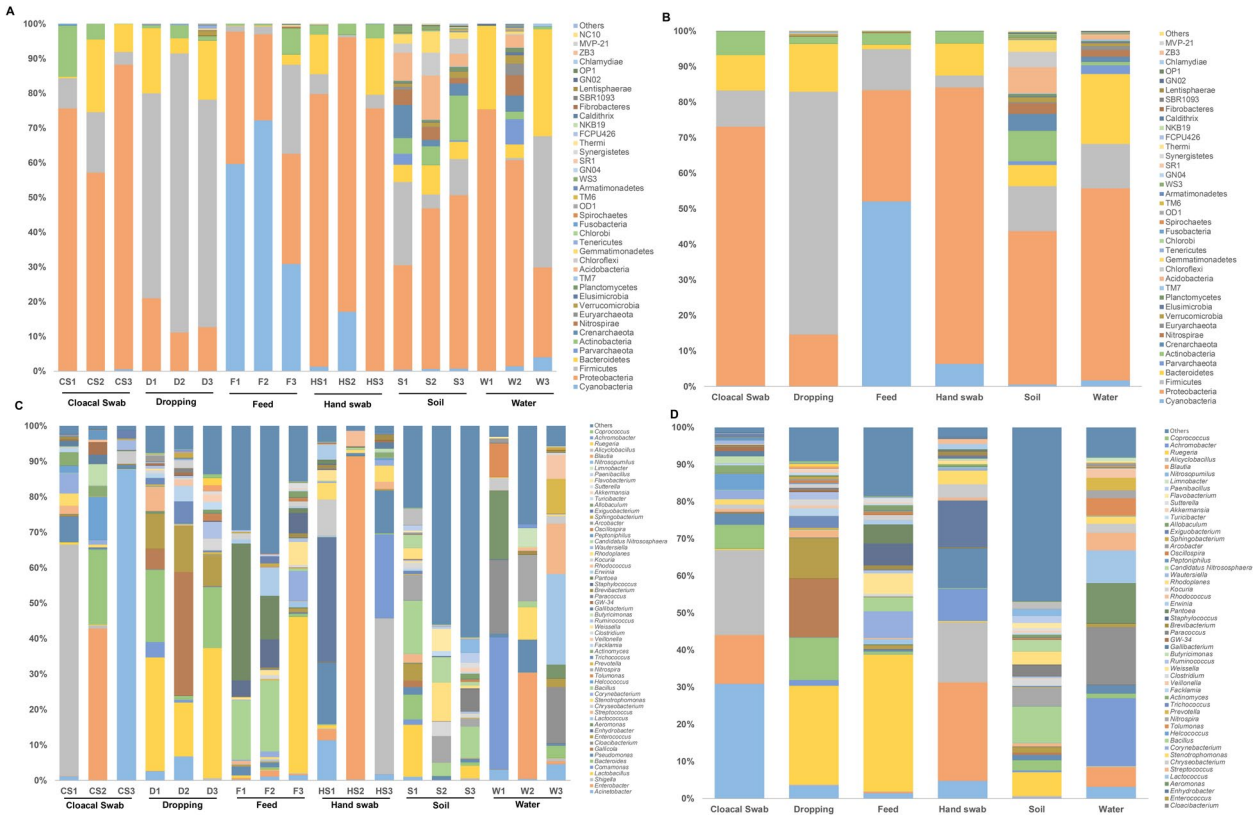

Fig. 4 Bar charts illustrating the relative abundance of microbial phyla and genera across different samples and sources. **A** and **B** show the relative abundance of the top 42 microbial phyla, with (**A**) representing phyla distribution across individual samples and (**B**) showing phyla according to sample sources, including Cloacal Swab, Dropping, Feed, Hand Swab, Soil, and Water. **C** and **D** depict the relative abundance of the top 61 microbial genera, with (**C**) focusing on genus-level composition by individual samples, and (**D**) presenting the distribution of genera grouped by sample sources, highlighting the microbial diversity in different environments

was significantly found in hand swabs (10.78%) and also detected in cloacal swabs (3.01%). *Corynebacterium* was predominantly identified in feed samples (7.25%) and minimally present in other samples. In contrast, *Actinomyces* showed low levels across all sample types, with the highest abundance observed in hand swabs (0.52%). *Butyricimonas* had a presence primarily in hand swabs (0.32%), while *Stenotrophomonas* was notably found in hand swabs (3.61%) and water (1.78%). The genus *Gallibacterium* was detected in hand swabs (0.68%) but was absent in soil and water samples. *Chryseobacterium* was found predominantly in water samples (2.44%) and had a low abundance in hand swabs (3.64%). Genera like *Lactobacillus* showed a high abundance in feed (36.89%) and cloacal swabs (0.33%), suggesting a strong association with dietary sources. *Streptococcus* was detected with higher abundance in droppings (1.88%) and water (4.82%), indicating its potential persistence in the gastrointestinal tract and aquatic environments. The genera *Enterococcus* and *Faecalibacterium* exhibited moderate abundance in droppings (10.78% and 0.46%, respectively) and hand swabs (0.95% and 0.03%, respectively). Other genera such as *Klebsiella* and *Bacillus* showed varying abundances across samples, with *Bacillus* reaching 9.84% in soil. The genus *Escherichia* was detected with a low abundance (0.02%) in water samples. Overall, the analysis highlighted the diverse microbial community structures across different environmental and biological samples, with certain genera showing strong associations with specific habitats.

Identification of genera of concern

The analysis of the genera of concern across various sources revealed distinct abundance patterns. *Enterobacter* was notably abundant in cloacal swabs (13.16%) and hand swabs (26.62%), indicating potential contamination or transmission routes. *Acinetobacter* showed significant presence in cloacal swabs (30.87%) and soil samples (0.33%), while *Shigella* was found primarily in hand swabs (16.12%) and cloacal swabs (22.89%), suggesting possible human-related transmission. *Comamonas* and *Pseudomonas* exhibited higher abundances

in water samples (18.37% and 10.78%, respectively), which may point to environmental reservoirs. Additionally, *Lactobacillus* was prevalent in feed (36.89%) and droppings (26.65%), indicating its potential role in gut health. Other genera, such as *Gallicola* (15.79% in droppings) and *Cloacibacterium* (15.67% in water), highlighted their presence in specific niches, further emphasizing the diversity and ecological roles of these microorganisms in the sampled environments (Fig. 5).

Functional pathway analysis

The KEGG Pathway analysis revealed several insights into the distribution of pathways across different sample types, including cloacal swabs, droppings, feed, hand swabs, soil, and water (Fig. 6). Pathways related to Cellular Processes demonstrated notable variability across samples. For instance, the *cell motility* pathway exhibited higher relative abundances in soil (2.05%) and hand swabs (2.50%) compared to cloacal swabs (1.68%), feed (1.50%), and water (2.53%). This suggests enhanced microbial motility within these environmental niches.

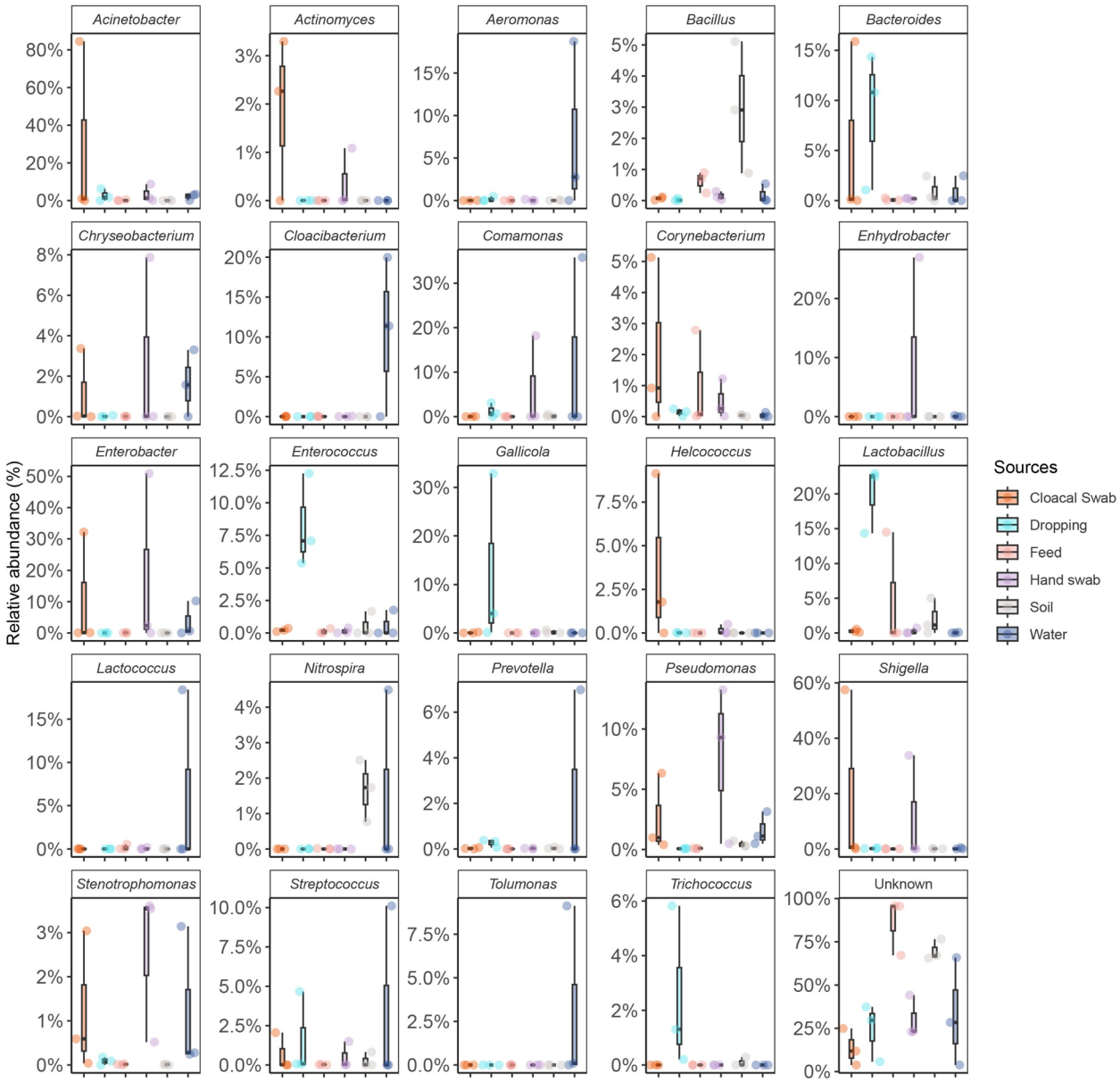

Fig. 5 Box plot illustrating the comparison of the top 25 genera across different sources

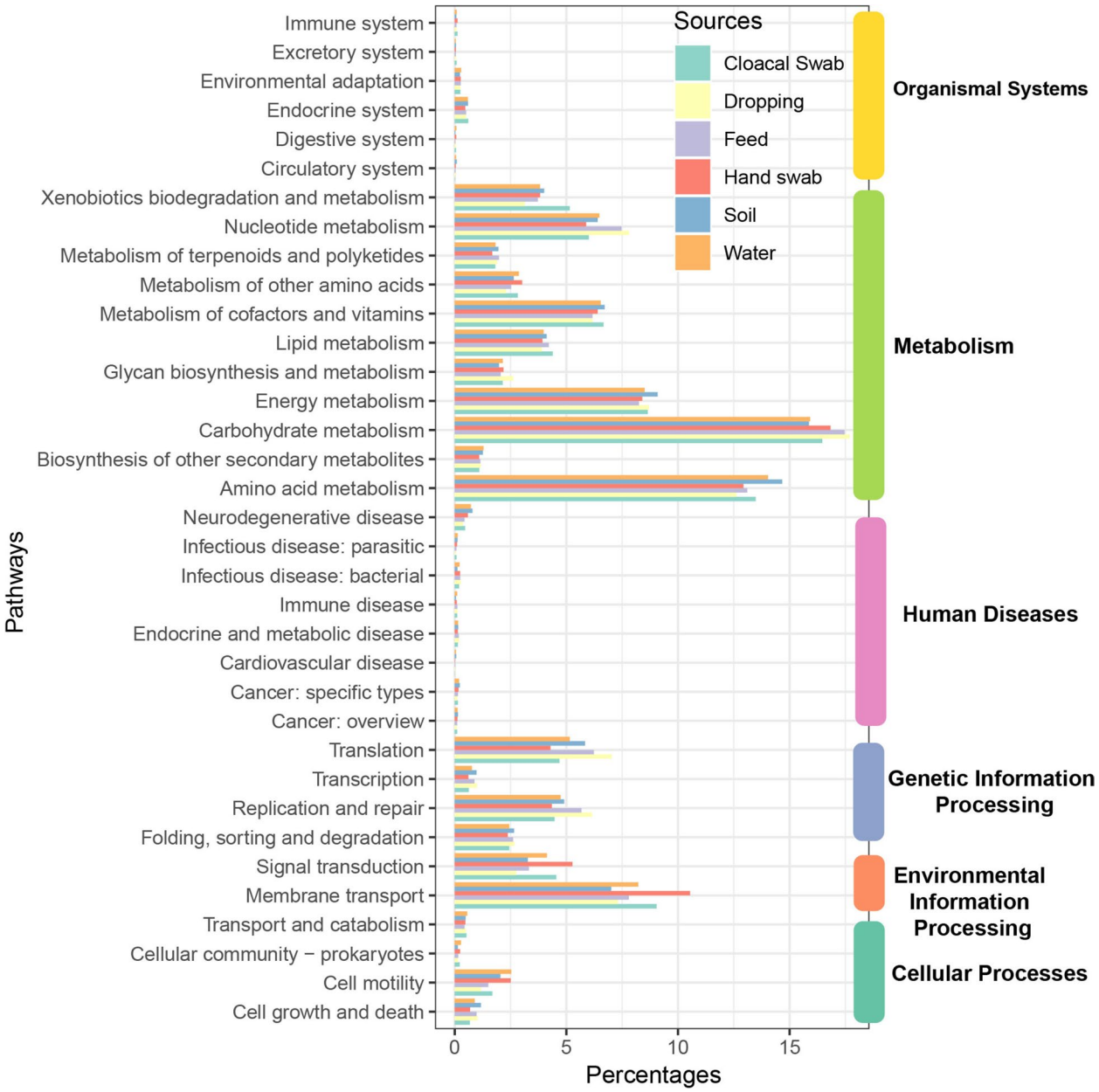

Fig. 6 Bar chart illustrating the relative abundances of KEGG pathways across six different sample types (cloacal swab, dropping, feed, hand swab, soil, and water). Pathways are grouped into five major KEGG Pathway Classes: Cellular Processes, Environmental Information Processing, Genetic Information Processing, Human Diseases, and Metabolism. Each bar represents the percentage contribution of a specific pathway within a given sample type, with pathways such as carbohydrate metabolism, membrane transport, and replication and repair showing higher relative abundances across multiple samples. The chart highlights the functional diversity of microbial communities, with variations in metabolic, cellular, and genetic processes between sample types, reflecting potential ecological adaptations of microbes to their respective environments

The *cell growth and death* pathway also showed elevated levels in soil (1.17%) and hand swabs (0.68%) compared to water (0.89%) and feed (0.98%), indicating dynamic microbial population shifts in these environments. Under Environmental Information Processing, the *membrane transport* pathway was dominant across all samples, with the highest values in hand swabs (10.54%) and cloacal swabs (9.04%), followed by water (8.22%) and feed (7.79%). This reflects that microbial communities in these environments may rely heavily on membrane transport mechanisms to interact with their surroundings. Similarly, *signal transduction* pathways were elevated in hand

swabs (5.27%) and water (4.13%), indicating that microbial communication is crucial in responding to environmental changes.

In Genetic Information Processing, the *replication and repair* pathway showed significantly higher abundance in droppings (6.15%) and feed (5.68%), compared to cloacal swabs (4.47%) and soil (4.91%). This suggests an active microbial genomic maintenance mechanism in these samples. The *translation* pathway also displayed higher percentages in droppings (7.03%) and feed (6.23%), reflecting increased protein synthesis for microbial growth in these environments. Pathways involved in Metabolism showed significant variations across sample types. *Carbohydrate metabolism* was particularly enriched in droppings (17.69%) and feed (17.46%), followed closely by water (15.92%) and soil (15.87%), suggesting that carbohydrate utilization is crucial for microbial survival in these habitats. Additionally, *amino acid metabolism* was abundant in all samples, with the highest percentages observed in soil (14.67%) and water (14.03%), further emphasizing the importance of nutrient cycling in these ecosystems. *Energy metabolism* also demonstrated notable levels, ranging from 8.24% in feed to 9.09% in soil, reflecting the critical role of energy production pathways across environments. For Human Diseases, pathways such as *infectious disease: bacterial* were present at low levels but showed slightly higher percentages in droppings (0.27%) and soil (0.24%), hinting at the potential presence of pathogenic bacteria in these samples. Similarly, pathways for *cancer* and *cardiovascular disease* were detected at lower levels, with *cancer: specific types* ranging from 0.12% in water to 0.23% in soil. Overall, this KEGG Pathway analysis highlighted the functional potential of microbial communities across different environmental samples, revealing key metabolic, genetic, and environmental response processes that govern microbial behavior in diverse ecological contexts. The relative abundances of specific pathways provided insights into how microbial communities may adapt and interact within their respective environments.

Concern of antimicrobial resistance pathways

The analysis of antimicrobial resistance (AMR) pathway abundance across various environmental samples revealed distinct patterns (Fig. 7). The 23S rRNA-methyltransferase exhibited the highest average relative abundance in Cloacal Swabs (8.96%), followed closely by Droppings (9.71%) and Water (9.27%), indicating a significant presence in fecal and aquatic environments. Similarly, the 16S rRNA-methyltransferase was notably abundant in Droppings (9.53%) and Feed (8.91%), suggesting its relevance in microbial communities associated with animal waste and nutrition. tRNA-methyltransferases showed moderate levels across all samples, with the highest in Droppings (6.14%). Noteworthy was the penicillin-binding protein, predominantly found in Droppings (5.55%) and Feed (4.53%), highlighting its potential role in antibiotic resistance. The multidrug efflux pump was particularly pronounced in Hand Swabs (4.48%), emphasizing its importance in human-related samples. Lower relative abundances were observed for acetyl-CoA C-acetyltransferase, with significant levels in Soil (4.36%). Other notable genes included the serine-type D-Ala-D-Ala carboxypeptidase and putative acetyltransferase, both of which had varying distributions across the samples, particularly in Droppings (2.53% and 1.20%, respectively). Several methyltransferases, such as release factor glutamine methyltransferase and phosphate acetyltransferase, were also present, with relative abundances ranging from 1.10% to 1.63% across the different samples. This highlights the diverse mechanisms employed by microorganisms to resist antimicrobial agents. The presence of beta-lactamase class A was minimal, especially in Cloacal Swabs (0.25%), indicating a relatively lower prevalence in comparison to other resistance genes. However, it was more prominent in Droppings (0.76%) and Feed (0.99%), which could point to selective pressures in these environments. The category labeled as Others encapsulated various antimicrobial resistance mechanisms, showing the highest combined abundance in the Hand Swab sample (19.09, 48.5%) and the lowest in Dropping (13.93, 35.4%) (Supplementary Data 1). Overall, the results underscore the diverse presence of antimicrobial resistance genes across different environmental samples, indicating potential sources and reservoirs for resistance traits in microbial populations.

The correlation analysis between the top 50 bacterial genera and the top 50 antimicrobial resistance (AMR) pathways revealed distinct patterns of association (Fig. 8). Several bacterial genera demonstrated strong positive or negative correlations with specific AMR pathways, suggesting potential roles in antimicrobial resistance dissemination. Among the genera, *Enterobacter, Acinetobacter, Shigella*, and *Pseudomonas* exhibited significant positive correlations with multiple AMR pathways, particularly those related to multidrug efflux pumps, beta-lactamase activity, and aminoglycoside resistance. These genera are well-known opportunistic pathogens commonly associated with nosocomial infections and multidrug resistance. Conversely, genera such as *Lactobacillus, Turicibacter*, and *Oscillospira* demonstrated negative correlations with key AMR pathways, indicating a potential protective or competitive role against antimicrobial resistance proliferation. This finding aligns with their known probiotic properties and contributions to

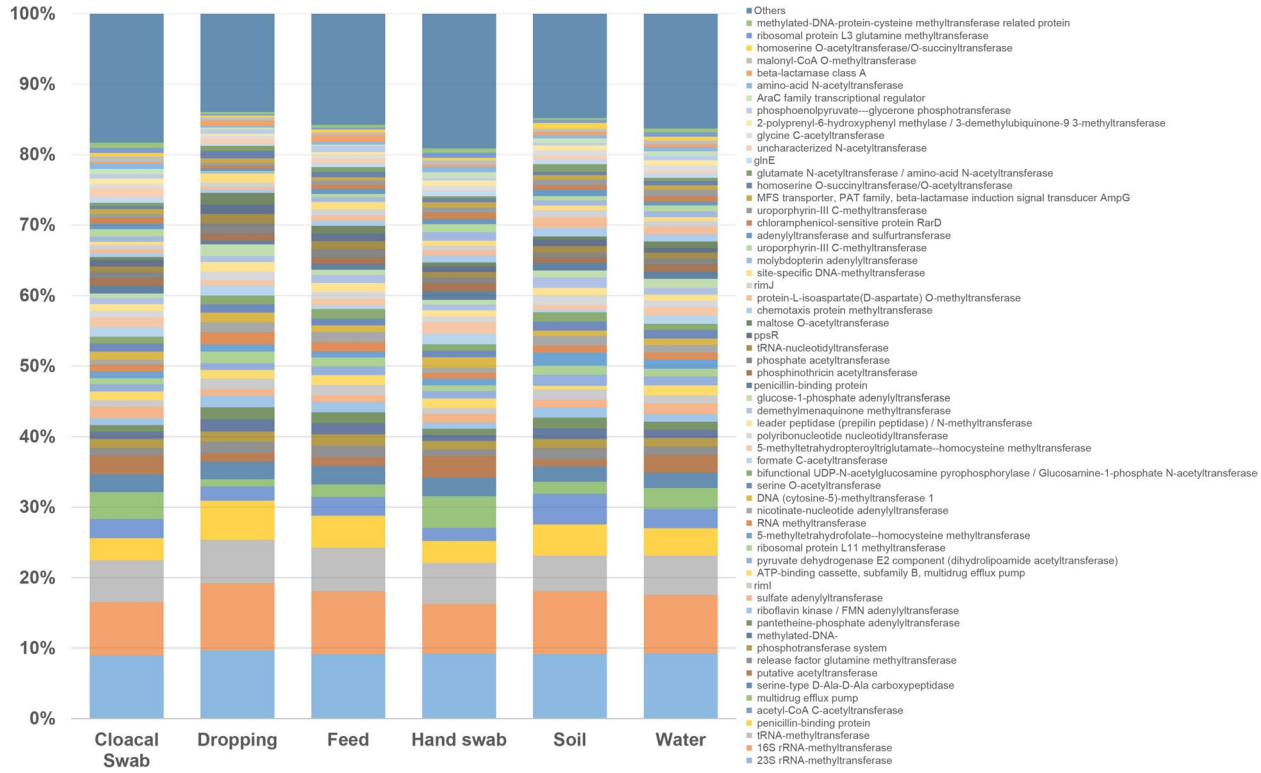

Fig. 7 Stacked bar plot illustrating the relative abundance of various antimicrobial resistance mechanisms across different environmental samples: Cloacal Swab, Dropping, Feed, Hand Swab, Soil, and Water. The y-axis indicates the percentage of antimicrobial resistance mechanisms' abundance, while the x-axis categorizes the sample types. Each segment within the bars is color-coded to represent specific resistance genes, highlighting the diversity of microbial enzymatic functions across these environments. This visualization emphasizes the prevalence of antimicrobial resistance in various ecological niches, contributing to our understanding of resistance dissemination

gut microbiome stability. Key AMR pathways, including 16S rRNA methyltransferase, penicillin-binding protein modifications, multidrug efflux pumps, and ATP-binding cassette transporters, showed strong associations with several pathogenic genera. In particular, the 16S rRNA methyltransferase pathway, linked to aminoglycoside resistance, exhibited a high positive correlation with *Enterobacter* and *Acinetobacter*, further highlighting their role in antibiotic resistance mechanisms. Notably, beta-lactam resistance pathways were significantly correlated with *Staphylococcus, Enterococcus*, and *Bacteroides*, which are known to harbor beta-lactamase genes. Additionally, acetyltransferase-related pathways, which contribute to resistance against aminoglycosides and chloramphenicol, were predominantly linked to *Shigella, Pseudomonas*, and *Aeromonas.* The results suggest that specific bacterial genera act as reservoirs for antimicrobial resistance determinants, potentially facilitating horizontal gene transfer within microbial communities. Understanding these associations can inform strategies to mitigate the spread of AMR and enhance targeted interventions in antimicrobial stewardship.

Discussion

Our study provides a comprehensive analysis of microbial diversity in poultry farm environments across Bangladesh, highlighting the influence of sample source and geographic location on microbial community composition and functional potential. The identification of distinct microbial communities in cloacal swabs, droppings, feed, hand swabs, soil, and water underscores the intricate relationship between microbial ecology and farm management practices. These findings align with previous research demonstrating the critical role of environmental factors in shaping microbial diversity and its implications for poultry health and productivity [34, 35].

Microbial diversity and pathogen dynamics

The predominance of Proteobacteria across sample types, particularly in cloacal swabs and water, reflects their essential role in nutrient cycling and ecosystem functioning [36]. However, the detection of potentially pathogenic genera, such as *Acinetobacter* and *Shigella*, raises significant concerns for poultry health and food safety. *Acinetobacter*, commonly associated with opportunistic

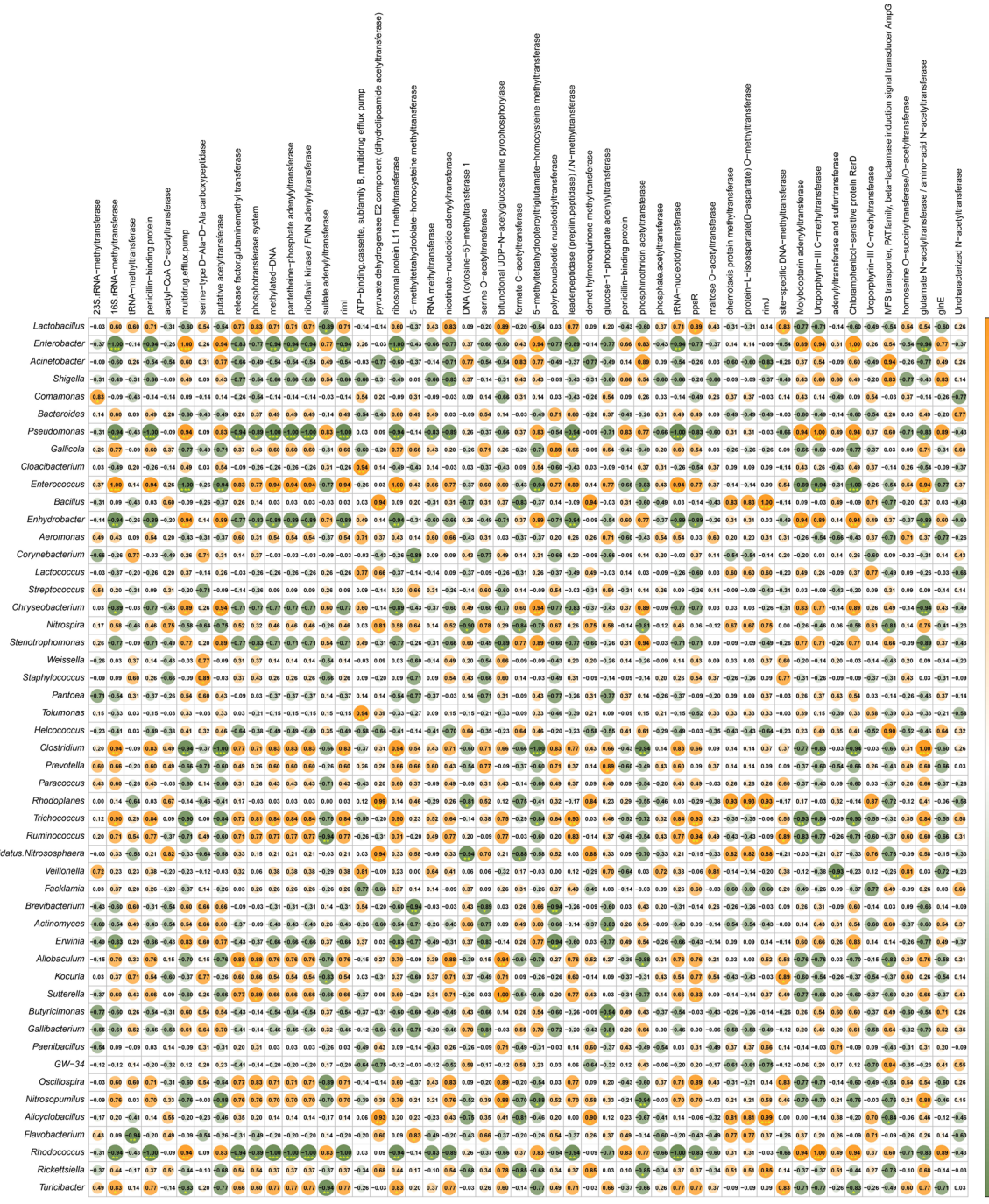

Fig. 8 The figure highlights the associations between dominant microbial genera and AMR pathways detected across different samples. Correlation matrix showing the relationships between the top 50 dominant bacterial genera (left) and a set of antibiotic resistance (AMR) genes (top). Spearman's correlation coefficients (r) are displayed, with vivid orange indicating positive correlations and moss green representing negative correlations. The intensity of the colors and the size of the circles reflect the strength of the correlations, where larger and darker circles denote stronger relationships. Significance levels are marked as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$

infections in stressed or immunocompromised birds, was prevalent in cloacal swabs and soil, suggesting its role as an environmental reservoir for pathogenic strains [37]. Similarly, the presence of *Shigella* in cloacal and hand swabs indicates potential fecal–oral transmission pathways, which could compromise poultry health and

increase the risk of zoonotic transmission [38]. These findings emphasize the need for enhanced biosecurity measures to mitigate pathogen spread and protect both animal and human health.

In contrast, the abundance of beneficial genera such as *Lactobacillus* and *Enterococcus* in feed and droppings highlights their potential role in promoting gut health and inhibiting pathogenic growth [39]. The strong association of *Lactobacillus* with feed suggests its utility as a probiotic, offering opportunities to improve poultry nutrition and health through dietary interventions [40]. These findings align with global trends in poultry microbiome research, which emphasize the importance of maintaining a balanced gut microbiome for optimal growth and disease resistance [41].

Antimicrobial Resistance (AMR) pathways and public health implications

The analysis of antimicrobial resistance (AMR) pathways revealed significant variations across sample types, with high abundances of 23S rRNA-methyltransferase in cloacal swabs, droppings, and water, and multidrug efflux pumps in hand swabs. These findings suggest that microbial communities in poultry farm environments are under selective pressure from antibiotic use, which is common in poultry farming for disease prevention and growth promotion [42]. The presence of AMR pathways in hand swabs further highlights the potential for human-mediated transmission of resistant bacteria, underscoring the need for stringent hygiene practices and regulatory oversight of antibiotic use in poultry production [43].

The correlation analysis between dominant bacterial genera and AMR pathways revealed strong positive associations between potentially pathogenic genera (*Enterobacter*, *Pseudomonas*, and *Acinetobacter*) and resistance mechanisms such as multidrug efflux pumps and beta-lactamase induction. These findings align with global concerns about the spread of multidrug-resistant bacteria in agricultural settings and their potential to enter the human food chain [44]. Conversely, beneficial genera such as *Lactobacillus* and *Streptococcus* exhibited negative correlations with certain AMR pathways, suggesting their potential role in mitigating antibiotic resistance through competitive exclusion or antimicrobial compound production [45]. These insights open new avenues for research into alternative strategies, such as probiotics, to control AMR in poultry farming systems.

Environmental and management implications

The observed variations in microbial composition across sample sources highlight the influence of environmental factors on microbial community dynamics. For example, the increased presence of Archaea in soil and water samples aligns with their known role in nutrient cycling and ecosystem functioning [46]. Similarly, the detection of *Pseudomonas* in hand swabs underscores the importance of biosecurity protocols to prevent cross-contamination and safeguard poultry health [47]. These findings emphasize the need for integrated management practices that address both microbial risks and environmental sustainability in poultry farming.

Limitations and future directions

While this study provides valuable insights into microbial diversity and AMR in poultry farms, several limitations should be acknowledged. The reliance on 16S rRNA amplicon sequencing, while effective for taxonomic profiling, limits the ability to infer functional potential and pathogenicity definitively. Predictive tools such as PICRUSt2, though useful, depend on reference genomes and may not capture environment-specific genes or pathways [48]. Future studies should incorporate multi-omics approaches, such as metagenomics and metatranscriptomics, to provide a more comprehensive understanding of microbial community dynamics and functional roles.

Additionally, the study focused on a limited number of sample sources and geographic locations within Bangladesh. Expanding the sampling strategy to include diverse environmental and geographical contexts would enhance the generalizability of the findings. Further research should also investigate the functional roles of microbial communities and their interactions, paving the way for innovative strategies to optimize poultry health and productivity while mitigating risks associated with pathogenic organisms and AMR.

Conclusion

This study provides a comprehensive analysis of microbial diversity, functional pathways, and antimicrobial resistance (AMR) in poultry farm ecosystems across Bangladesh. By examining cloacal swabs, droppings, feed, hand swabs, soil, and water samples, we identified distinct microbial communities shaped by sample source and geographic location. Droppings exhibited the highest microbial richness, while feed samples showed the lowest diversity, underscoring the influence of environmental factors on microbial composition. Proteobacteria emerged as the dominant phylum, with pathogenic genera such as *Enterobacter*, *Acinetobacter*, and *Shigella* prevalent in cloacal and hand swabs, highlighting potential contamination and zoonotic risks. Conversely, beneficial genera like *Lactobacillus* and *Enterococcus* were abundant in feed and droppings, suggesting roles in gut health and nutrient cycling. Functional pathway analysis revealed active metabolic processes, particularly carbohydrate

and amino acid metabolism, in droppings and feed, reflecting the critical role of microbial communities in nutrient utilization. The widespread presence of AMR pathways, such as 23S rRNA-methyltransferase and multidrug efflux pumps, further emphasizes the risks associated with antimicrobial resistance in poultry farming. Pathogenic genera showed strong correlations with AMR pathways, indicating their potential as reservoirs for resistance genes. These findings underscore the importance of microbial ecology in poultry health and productivity, as well as the need for improved management practices and biosecurity measures. By addressing contamination risks, enhancing hygiene protocols, and regulating antibiotic use, the poultry industry in Bangladesh can mitigate the spread of pathogens and AMR, ensuring safer and more sustainable poultry production. Future research should focus on functional validation of microbial roles and the development of targeted interventions, such as probiotics, to optimize poultry health and reduce reliance on antibiotics.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03970-0.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
This study has received a generous instrument support from the Department of Microbiology, Jashore University of Science and Technology and we thank the farm owner's for supporting sample collection and giving information for data. We are grateful to the Ministry of Education, Bangladesh for research Grant.

Authors' contributions
AY designed the study, collected samples and data, extracted DNA, performed laboratory work, interpreted the data and drafted the manuscript. MSR contributed to study design, performed bioinformatics analysis, visualized figures, interpreted results and finalized the manuscript. SMK, MMA, and MEKM analyzed the data and contributed to drafting the manuscript. HA assisted with study design and critically reviewed the manuscript. MS and AB designed and supervised the study and critically reviewed and finalized the manuscript.

Funding
We Thank the Ministry of Education, Government of the People's Republic of Bangladesh for funding the research project (Project No. LS20222304).

Data availability
"The data that support the findings of this study are openly available in NCBI BioProject at PRJNA1179713."

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh. [2]Present Department of Pathobiology, Gazipur Agricultural University, Gazipur 1706, Bangladesh. [3]Department of Microbiology, Jashore University of Science and Technology, Jashore, Bangladesh.

Received: 13 November 2024 Accepted: 16 April 2025
Published online: 22 May 2025

References

1. Davis MF, Price LB, Liu CMH, Silbergeld EK. An ecological perspective on US industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments. Cur Opinion Microbiol. 2011;14(3):244–50.
2. Ricke SC, Richardson K, Dittoe DK. Formaldehydes in feed and their potential interaction with the poultry gastrointestinal tract microbial community–a review. Frontiers in Veterinary Science. 2019;6:188.
3. Kogut MH. The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol. 2019;250:32–40.
4. Wittwer M, Keller J, Wassenaar T, Stephan R, Howald D, Regula G, et al. Genetic diversity and antibiotic resistance patterns in a Campylobacter population isolated from poultry farms in Switzerland. Appl Environ Microbiol. 2005;71(6):2840–7.
5. Hakeem MJ, Lu X. Survival and control of Campylobacter in poultry production environment. Front Cell Infect Microbiol. 2021;10: 615049.
6. Garcia J, Kao-Kniffin J. Microbial group dynamics in plant rhizospheres and their implications on nutrient cycling. Front Microbiol. 2018;9:1516.
7. Bryan FL, Doyle MP. Health risks and consequences of Salmonella and Campylobacter jejuni in raw poultry. J Food Prot. 1995;58(3):326–53.
8. Hazards EPoB, Koutsoumanis K, Allende A, Álvarez-Ordóñez A, Bolton D, Bover-Cid S, et al. Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. Efsa J. 2021;19(6):e06651.
9. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev. 2011;75(4):583–609.
10. Sibanda N, McKenna A, Richmond A, Ricke SC, Callaway T, Stratakos AC, et al. A review of the effect of management practices on Campylobacter prevalence in poultry farms. Front Microbiol. 2018;9:2002.
11. Bindari YR, Moore RJ, Van TTH, Hilliar M, Wu S-B, Walkden-Brown SW, et al. Microbial communities of poultry house dust, excreta and litter are partially representative of microbiota of chicken caecum and ileum. PLoS ONE. 2021;16(8): e0255633.
12. Michaels B, Keller C, Blevins M, Paoli G, Ruthman T, Todd E, et al. Prevention of food worker transmission of foodborne pathogens: risk assessment and evaluation of effective hygiene intervention strategies. Food Service Technology. 2004;4(1):31–49.
13. Hossain MJ, Attia Y, Ballah FM, Islam MS, Sobur MA, Islam MA, et al. Zoonotic significance and antimicrobial resistance in Salmonella in poultry in Bangladesh for the period of 2011–2021. Zoonoticdis. 2021;1(1):3–24.
14. Alfonsi C, Di Pietro F, Papa FT, Gabrielli F. Decoding Microbial networks: an insight into 16S rRNA and whole genome sequencing approaches in Metagenomic studies. J Biomed Res Environ Sci. 2023;4(10):1443–6.
15. Shin H, Lee E, Shin J, Ko S-R, Oh H-S, Ahn C-Y, et al. Elucidation of the bacterial communities associated with the harmful microalgae Alexandrium tamarense and Cochlodinium polykrikoides using nanopore sequencing. Sci Rep. 2018;8(1):5323.
16. Ortiz-Estrada ÁM, Martínez-Porchas M, Martínez-Córdova LR, Vargas-Albores F, Burgos-Hernandéz A, Scheuren-Acevedo SM, et al. Bacterial communities and predicted nitrogen metabolism of heterotrophic-and probiotic-based biofilms used for super-intensive indoor shrimp culture. Aquac Res. 2021;52(1):334–44.
17. Yin Q, Fu B, Li B, Shi X, Inagaki F, Zhang X-H. Spatial variations in microbial community composition in surface seawater from the ultra-oligotrophic center to rim of the South Pacific Gyre. PLoS ONE. 2013;8(2): e55148.
18. Andrews, S. FastQC: A quality control tool for high throughput sequence data. Cambridge: Babraham Bioinformatics, Babraham Institute . (2010).

19. Patel RK, Jain M. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS ONE. 2012;7(2): e30619.
20. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20. https://doi.org/10.1093/bioinformatics/btu170.
21. Del Fabbro C, Scalabrin S, Morgante M, Giorgi FM. An extensive evaluation of read trimming effects on Illumina NGS data analysis. PLoS ONE. 2013;8(12): e85024.
22. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7. https://doi.org/10.1038/s41587-019-0209-9.
23. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4: e2584.
24. Ziemski M, Wisanwanichthan T, Bokulich NA, Kaehler BD. Beating naive bayes at taxonomic classification of 16S rRNA gene sequences. Front Microbiol. 2021;12: 644487.
25. Liu G, Li T, Zhu X, Zhang X, Wang J. An independent evaluation in a CRC patient cohort of microbiome 16S rRNA sequence analysis methods: OTU clustering, DADA2, and Deblur. Front Microbiol. 2023;14:1178744.
26. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8(4): e61217.
27. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara R, et al. Package 'vegan'. Commun Ecol Package version. 2013;2(9):1–295.
28. Kassambara, A. R Graphics Essentials for Great Data Visualization: 200 Practical Examples You Want to Know for Data Science. STHDA. 2017.
29. Wickham H. ggplot2. Wiley interdisciplinary reviews: computational statistics. 2011;3(2):180–5.
30. Shetty, S. A., Lahti, L., de Vos, W. M., & Smidt, H. microbiomeutilities: An R package for utilities to guide in-depth marker gene amplicon data analysis. Ecophysiological insights into the human intestinal microbiota: from single strains to defined consortia, 2018;95.
31. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38(6):685–8.
32. Sun S, Jones RB, Fodor AA. Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome. 2020;8:1–9.
33. Yang C, Mai J, Cao X, Burberry A, Cominelli F, Zhang L. ggpicrust2: an R package for PICRUSt2 predicted functional profile analysis and visualization. Bioinformatics. 2023;39(8):btad470.
34. Islam MS, Hossain MJ, Sobur MA, Punom SA, Rahman AT, Rahman MT. A Systematic Review on the Occurrence of Antimicrobial-Resistant Escherichia coli in Poultry and Poultry Environments in Bangladesh between 2010 and 2021. Biomed Res Int. 2023;2023(1):2425564.
35. Aruwa CE, Pillay C, Nyaga MM, Sabiu S. Poultry gut health–microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. Journal of Animal Science and Biotechnology. 2021;12:1–15.
36. Howard A, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii: an emerging opportunistic pathogen. Virulence. 2012;3(3):243–50.
37. Bagamian KH, Anderson JD IV, Blohm G, Scheele S. Shigella and childhood stunting: Evidence, gaps, and future research directions. PLoS Negl Trop Dis. 2023;17(9): e0011475.
38. Hills RD, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut microbiome: profound implications for diet and disease. Nutrients. 2019;11(7):1613.
39. Halder N, Sunder J, De AK, Bhattacharya D, Joardar SN. Probiotics in poultry: a comprehensive review. The Journal of Basic and Applied Zoology. 2024;85(1):23.
40. Song B, Fang J, Yu Z, Liu Z, Li N, Pena R, et al. The development of biological soil crust along the time series is mediated by archaeal communities. Geoderma. 2024;449: 117022.
41. Verdial C, Carneiro C, Machado I, Tavares L, Almeida V, Oliveira M, et al. Controlling bacteriological contamination of environmental surfaces at the biological isolation and containment unit of a veterinary teaching hospital. Ir Vet J. 2021;74:1–10.
42. Marmion M, Ferone M, Whyte P, Scannell A. The changing microbiome of poultry meat; from farm to fridge. Food Microbiol. 2021;99: 103823.
43. Gržinić G, Piotrowicz-Cieślak A, Klimkowicz-Pawlas A, Górny RL, Ławniczek-Wałczyk A, Piechowicz L, et al. Intensive poultry farming: A review of the impact on the environment and human health. Sci Total Environ. 2023;858: 160014.
44. Gupta CL, Blum SE, Kattusamy K, Daniel T, Druyan S, Shapira R, et al. Longitudinal study on the effects of growth-promoting and therapeutic antibiotics on the dynamics of chicken cloacal and litter microbiomes and resistomes. Microbiome. 2021;9:1–19.
45. Shehata AA, Yalçın S, Latorre JD, Basiouni S, Attia YA, Abd El-Wahab A, et al. Probiotics, prebiotics, and phytogenic substances for optimizing gut health in poultry. Microorganisms. 2022;10(2):395.
46. Panyako PM, Ommeh SC, Kuria SN, Lichoti JK, Musina J, Nair V, et al. Metagenomic Characterization of Poultry Cloacal and Oropharyngeal Swabs in Kenya Reveals Bacterial Pathogens and Their Antimicrobial Resistance Genes. International Journal of Microbiology. 2024;2024(1):8054338.
47. Taylor-Bowden T, Bhogoju S, Khwatenge CN, Nahashon SN. The Impact of Essential Amino Acids on the Gut Microbiota of Broiler Chickens. Microorganisms. 2024;12(4):693.
48. Zhang T, Nickerson R, Zhang W, Peng X, Shang Y, Zhou Y, et al. The impacts of animal agriculture on One Health—Bacterial zoonosis, antimicrobial resistance, and beyond. One Health. 2024;18:100748.

Publisher's Note