A longitudinal study of chicken caecal microbiota focused on microbial diversity and the occurrence of AMR genes

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Introduction

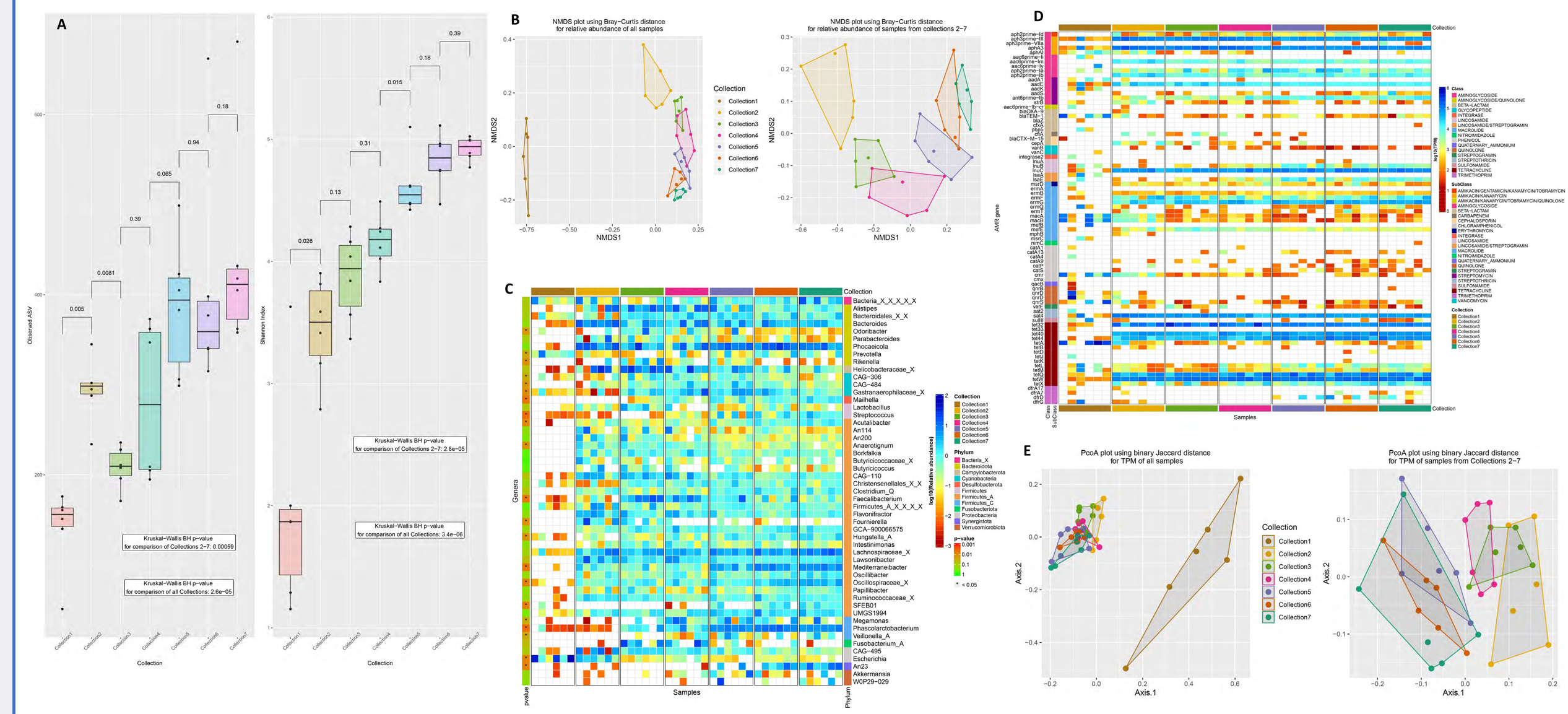
The microbiota in caecum of birds plays a key role in chicken health and performance, influencing digestion and absorption of nutrients, and contributing to defense against colonization by invading pathogens. Major colonization of the chicken caecum occurs after hatch and this, along with subsequent microbiota composition and activity, are influenced by numerous host and environmental factors. The present study was conducted to track the structure of caecal microbiota during the life-cycle of broiler chicken. Additionally, the study also reports on the AMR gene profile of caecal microbiota.

Methodology

- Caecal content samples from **six birds per week** (1 from different pan) were collected on **0th day (Collection-1), 8th day (Collection-2), 15th day (Collection-3)** and **so on till 43rd day (Collection-7)** making total of 42 samples.
- **Metagenomic DNA** was extracted from the samples. **V3-V4 region of 16S rRNA** gene was targeted (341F and 785R primers) and sequenced on Illumina MiSeq using 250x2 v2 chemistry.
- Same metagenomic DNA was used **to target 493 genes using 834 amplicons** part of customised AmpliSeq AMR panel from Illumina and sequenced on Illumina MiSeq using 250x2 v2 chemistry.
- 16S rRNA gene amplicon data was analysed using **DADA2 v1.14.1** using default pipeline and taxonomy was assigned using **GTDB r95** database.
- Ampliseq AMR data was quality filtered using **Prinseq-lite** script and pair reads were merged using **PANDAseq**. Merged data was **mapped to custom reference** file created from targeted amplicon sequences using **BWA-mem**. Sam files were processed to **calculate per reference sequence reads mapped, average coverage and horizontal coverage**. Calculated data was imported and further analysed in R. **Transcripts per million (TPM)** was calculated from number of reads mapped and length of reference. TPM was used for all further analysis.

Result

- Least diversity was observed in the 0th day samples with **significantly increasing diversity with increase in age** (Figure A).
- **Collection-1 samples** were also the **most distinct** from all other samples. However, a **horse-shoe like pattern** was observed across samples from all collection (Figure B).
- Firmicutes_A and Bacteroidota phyla were the most abundant phyla in Collections 2 to 7. *Clostridium*, *Escherichia* and *Niameybacter* were the most abundant genera in Collection 1, while *Alistipes*, *Phocaeicola*, *Bacteroides*, *Phascolarctobacterium* and some unknown organisms from *Lachnospiraceae* and *Helicobacteraceae* family were the most abundant. Total **71 genera differed significantly** among Collections 2 to 7 (Figure C).
- Out of total 493 genes/targets, **172 were detected** from all samples including **82 with reads >10**. Most of these reads were assigned to targets giving resistance to **tetracycline** and **Aminoglycoside**. In particular, *aph2''-Id* and *aphA3* (Aminoglycoside); *lnuC* (Lincosamide); *sat4* (Streptothricin); *tet32*, *tet40*, *tet44*, *tetQ* and *tetW* (Tetracycline) genes were overly abundant (Figure D).
- AMR gene profile of Collection-1 was significantly different than other samples. Furthermore, separate clusters were observed for Collections 2 to 4 and Collections 5 to 7, differentiating finisher diet samples from starter and pre-starter diets (Figure E).



A: Alpha diversity metrics Observed ASVs and Shannon Index plotted against collections. Subsequent collections are compared using Wilcoxon-test, while multi-group comparisons are done using Kruskal-Wallis test.
B: NMDS plot prepared using Bray-Curtis distance of relative abundance of all ASVs.
C: Heatmap showing 50 most abundant genera (considering collections 2 to 7). Benjamini-Hochberg adjusted p-values for comparison of abundance across collections 2-7 is presented in the left-side bar.
D: Heatmap showing distribution of AMR genes.
E: PCoA plot of the distribution of binary Jaccard distance (presence/absence) of TPM of AMR genes.

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