Variation in antimicrobial resistance gene occurrence in the caecal microbiota of broiler chickens fed antibiotics or probiotics

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Introduction

- Poultry production is one of the fastest growing industries worldwide.
- Over 70% of antimicrobials produced globally are used as growth promotor in food animals.
- Chicken caecal microbiomes have been linked to resistance to pathogen colonization, as well health and growth performance, but the widespread use of antibiotics in production has raised concern of antimicrobial resistance (AMR).

Objective

- Comparing the profile of AMR genes in the caecal microbiota of Cobb400 broiler chickens reared without and with feed additives (antibiotics and probiotics).

Methodology

- Total 540 hatched day-old Cobb400 commercial broiler chicks were part of the study.
- Eighteen pens of 30 chickens were divided into three treatment groups. T1 (control) group: fed commercial standard diet without any feed additives, T2 group: supplemented with antibiotics and T3 group: supplemented with multistrain probiotic.
- Caecal content was collected each week from one bird per pen for seven weeks. Metagenomic DNA extracted from caecal contents of last two collections (35th and 42nd day) was surveyed for the occurrence of 493 AMR genes using an AMR AmpliSeq Panel with Illumina MiSeq sequencing.
- 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 using pileup.sh script part of BBMap suite to calculate per reference sequence reads mapped, average coverage and horizontal coverage.
- Calculated data was imported and further analyzed in R. Transcripts per million (TPM) was calculated from number of reads mapped and length of reference. TPM was used for all further analysis.

Results

- In total 122 gene targets were detected from the 35th and 42nd day of production. Tetracycline resistance sequences were most abundant (*tet32, tet40, tet44, tetQ and tetW*), followed by sequences linked to aminoglycoside (*aph3prime-III and aphA3*) and lincosamide (*lnuC*) resistance (Figure A & B).
- However, at the higher level, only the occurrence of sequences associated with streptrothricin resistance class in 35th day collection was significantly different ($p < 0.09$) with higher abundances in T2.
- Additionally, 5 genes (*tetL, sat4, dfrG, tetM* and *dfrD*) and 4 genes (*aphAI, macB, cmr* and *mefE*) showed significant differences (p-value < 0.05) among three treatments in Collection-6 and Collection-7, respectively (Figure C & D).

Discussion & Conclusion

- Findings indicate that the tested antibiotic and probiotic formulations not elicit significant changes in the AMR gene profile of cecal microbiota.
- Overall, significant differences were observed for few genes in AMR profiles even when antibiotics were supplemented in the diet of birds.

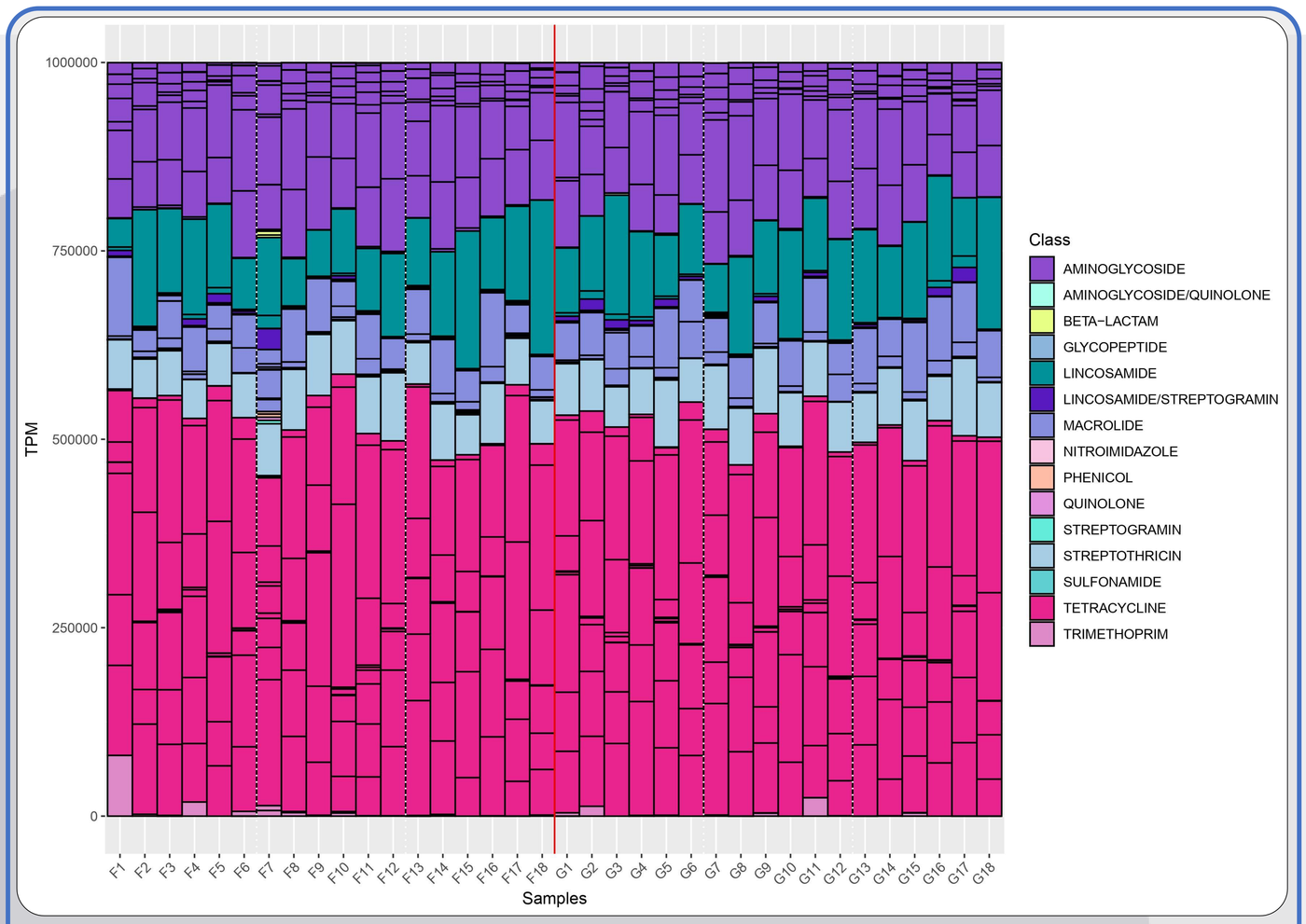

Figure A: Stacked bar plot showing TPM of each gene/target coloured by the AMR class (In samples F-represent 35th day collection while G-represent 42nd day collection; within that 1-6 samples represent T1 group (Control), 7-12 samples represent T2 group (Antibiotic), 13-18 samples represent T3 group (Probiotic).

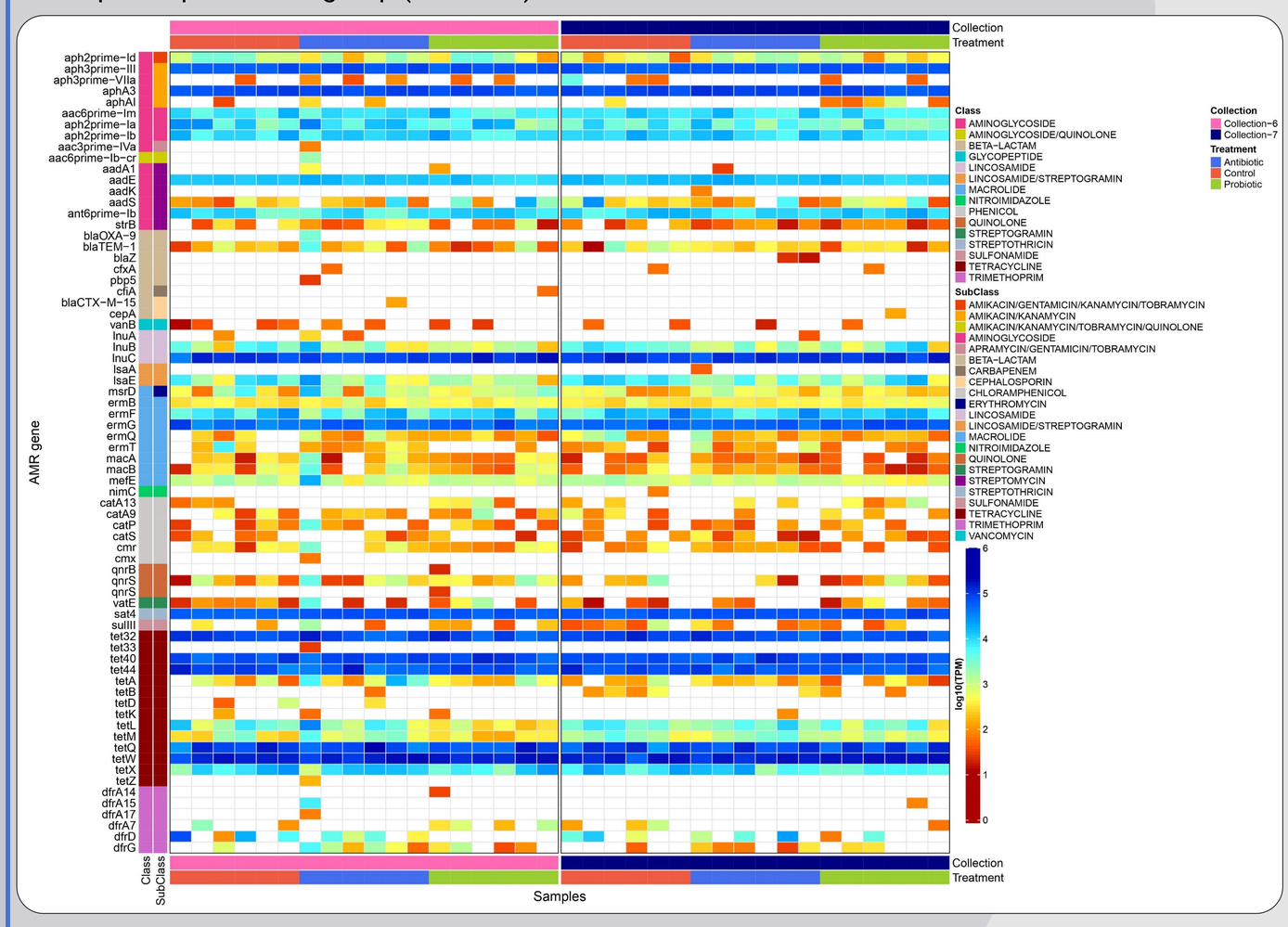

Figure B: Heatmap showing distribution of AMR genes.

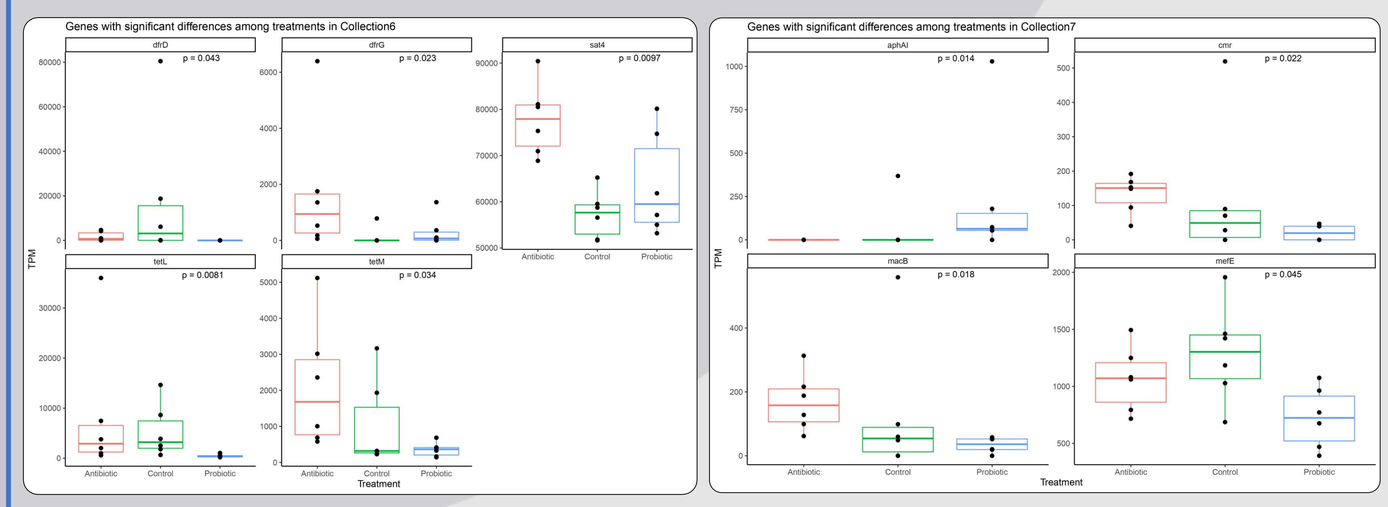

Figure C: Boxplot showing TPM of AMR genes with significant differences among treatments in collection-6 (35th day).

Figure D: Boxplot showing AMR genes with significant differences among treatments in collection-7 (42nd day).

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