**Plasmid-Mediated Antibiotic Resistance Dynamics in Broiler Chickens Revealed by Long-Read Sequencing**

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The widespread use of antibiotics in human and veterinary medicine has accelerated antimicrobial resistance (AMR), primarily through horizontal gene transfer. This process allows bacteria to exchange antibiotic resistance genes (ARGs) via mobile genetic elements like plasmids. Antibiotic use creates ARG reservoirs in bacterial populations, promoting the spread of resistance and contributing to multidrug-resistant pathogens. Intensive poultry farming has notably contributed to AMR, with excessive antibiotic use promoting the accumulation of ARGs in the chicken gut microbiome. These ARGs, often carried on plasmids, can transfer between bacteria, including pathogens, posing a risk to human health via food or environmental exposure.

In this study, long-read sequencing was used to analyze the plasmidome and resistome in 12 fecal samples collected from three chicken barns on a commercial farm. All chickens received enrofloxacin during the first days of life, with one barn was additionally treated with sulfamethoxazole/trimethoprim. For comparison, short-read metagenomic sequencing was also performed on the same samples.

The analysis revealed ARGs associated with resistance to 26 antibiotic classes, highlighting the diversity of the poultry resistome. Strong genetic links were observed between specific plasmids and ARGs, with MOBP plasmids frequently associated with fluoroquinolone resistance. Temporal trends indicated progressive mobilization of resistance genes, suggesting an increasing potential for horizontal gene transfer. While fluoroquinolone resistance expanded, diaminopyrimidine resistance remained stable despite treatment. Most ARGs were carried on small plasmids (2.6 to 47.6 kb); several exhibited high similarity to plasmids from other bacterial species, indicating potential for cross-species gene transfer, with possible transmission from poultry to humans through shared agricultural or environmental spaces. Most ARGs were found on small plasmids ranging from 2.6 to 47.6 kb. Several plasmids showed high sequence similarity to those from other bacterial species, indicating a potential for cross-species transfer and possible transmission from poultry to humans through shared agricultural or environmental pathways.

These findings provide new insights into the dynamics of the poultry gut microbiome and antimicrobial resistance, emphasizing the central role of plasmids in gene mobilization. The results underscore the importance of sustained monitoring and targeted strategies to mitigate the spread of antimicrobial resistance in both agricultural and clinical environments.

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