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Precise detection of antibiotic resistance genes in chicken microbiota

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Antibiotic resistance genes (ARGs) presence is largely documented in gut microbiomes of farm animals and represent a major risk for animal and human health but the routes and mechanisms of their dissemination are yet only partially understood. At the epidemiological level, it would be interesting to better understand the respective roles of contamination from the environment and transmission between generations of animals. These transmission events, at the genetic level, may be mediated by horizontal transfer of ARGs and clonal propagation of ARG harbouring bacterial lineages. To study these questions, an experiment was conducted aiming at following ARGs by shotgun metagenomics across 3 generations of broiler chicken in two separate buildings.

The study of ARG in metagenomic samples is challenging, because of sequencing errors, short read length, and variable abundance of genes, which suggest that specific methods may be needed to properly identify ARG flows. Moreover, preliminary results have shown that several haplotypes can co-exist for each gene, and that these haplotypes may be shared or not between samples. Analyzing this micro-evolutionary diversity is the key idea of our work. The identification of haplotypes in metagenomic data is a challenge that has been recently addressed with methods to identify strains relying on SNP frequencies in selected core genome marker genes (such as ConStrains[1], or DESMAN[2]), or with methods using reads as a phasing information to resolve haplotypes on individual genes (Hansel & Gretel [3]).

In order to accurately detect ARG flows between samples, we are working on a new workflow based on aligning metagenomic reads on a non redundant database of reference ARG sequences (Resfinder4.1 [4] clustered at 95% of identity) with BWA-MEM, and stringent quality filters are applied on reads and alignments using samtools. Nucleotide frequencies at each position of these reference sequences are then computed and statistics (number of polymorphic positions, number of alleles...) are recorded in order to quantify the diversity in ARGs within samples and the distance between samples. Our first results indicate a huge variability between genes in terms of number of polymorphic positions and number of haplotypes. Future steps will consist in identifying shared ARG haplotypes between samples by using approaches derived from those used for strain resolution to resolve the different haplotypes and determine their abundances in each sample, or by trying to infer ARG fluxes by comparing samples based on diversity and distance statistics only, without haplotype resolution.

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