Optimization of AMR sequencing and analysis pipelines: Pilot study on Hub samples

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AMR Threats: Global Problem ! Local Intervention ! Global Impact !

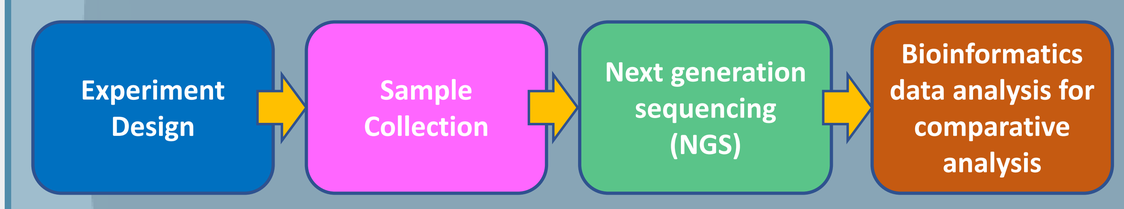
AMR occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death.

Introduction

Antibiotics are used to fight bacterial infections. However, antibiotic resistance is considered to be one of the major healthcare challenges in recent times. Recognizing its significance, it is important to assess the occurrence of genetic determinants of drug resistance in medical, veterinary and public health settings in order to understand risks of transmission and treatment failure. With the advancement in sequencing technology, culture independent methods have gained popularity among the researchers across the globe.

Methods

Twelve healthy broiler chickens (Cobb 400, 37 days old) were collected from the Central Poultry Research Station of Anand Agricultural University, Anand, Gujarat. After genomic DNA isolation from caecum, all samples were processed in parallel for the two sequencing technologies (Illumina MiSeq and Ion Torrent). Subsequently, following a common bioinformatics workflow to define the occurrence and abundance of AMR gene sequences.



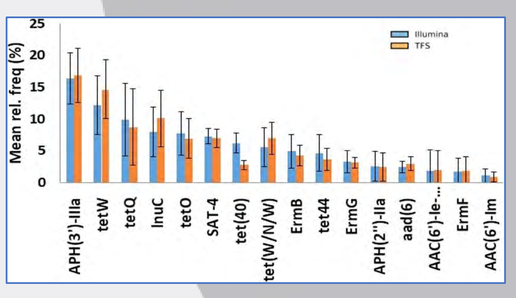


Fig. 1: The relative sequencing read abundance of AMR genes amplified from chicken caeca microbial populations with ≥1% abundance within equivalent Illumina MiSeq and Ion Torrent platform.

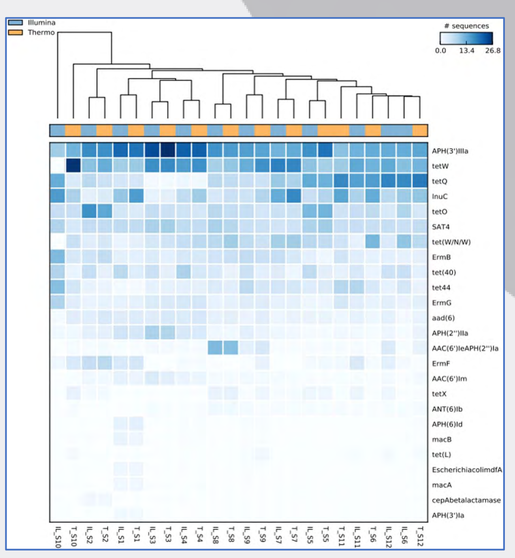


Fig. 2: Heatmap demonstrating the abundance of the top 25 AMR genes in 12 chicken caeca samples.

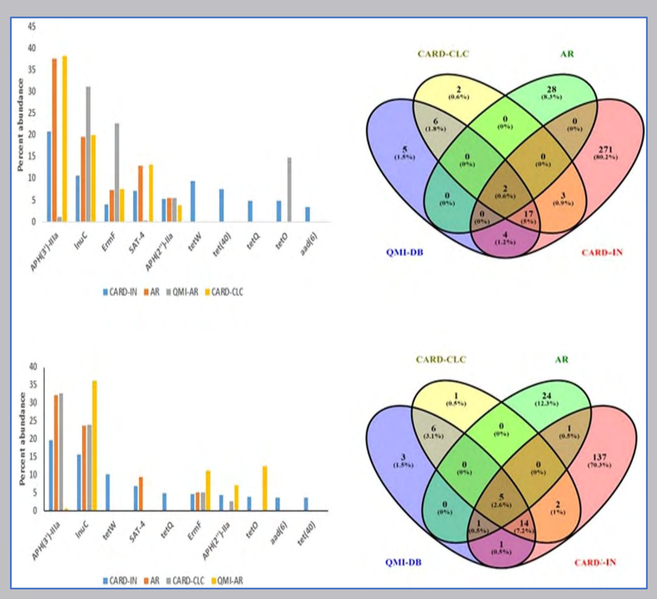


Fig. 3: Comparison of AMR gene assessment of amplicon sequencing generated using Illumina MiSeq and Ion Torrent. Databases included were (CARD-CLC- CARD database present in CLC genomic workbench microbial genomic module, AR-Antibiotic resistance database, QMI-DB-QIAGEN microbial Insight-AR, CARD-IN-CARD database downloaded from CARD site and run local server.

Objectives

The aim of the present study was to

- Profiling of the AMR genes using NGS technology in the poultry food chain and compare the Illumina MiSeq and Ion Torrent S5 Plus sequencing platforms for use with the Ion AmpliSeq AMR Research Panel.

Results

In total ~15M reads were obtained using the Ion Torrent S5 Plus platform in a single FastQ file, representing approximately 1M reads per sample with an average read length of 200bp. While, 4.18M reads were produced for the same samples using Illumina MiSeq, representing 0.2M reads per sample with an average read length of 185 bp.

The *APH (3')-IIIa* gene (Fig.1 & 2) was found to be most abundant in both the platforms followed by *tetW* and *tetQ*. The occurrence of only nine genes was found to be significantly different between the sequencing platforms. Out of these nine genes, *tet(40)* was found to be most variable (4%). Different databases were also compared for ARGs (Fig.3).

Taxonomically, *Campylobacter coli* CVM N29710 (Fig. 4) was the most abundant organism identified, followed by *Bacteroides fragillis*. Only *Staphylococcus epidermidis* was found to be significantly differently represented between the platforms (q-value (corrected) = 0.001) (Abundance <0.0014).

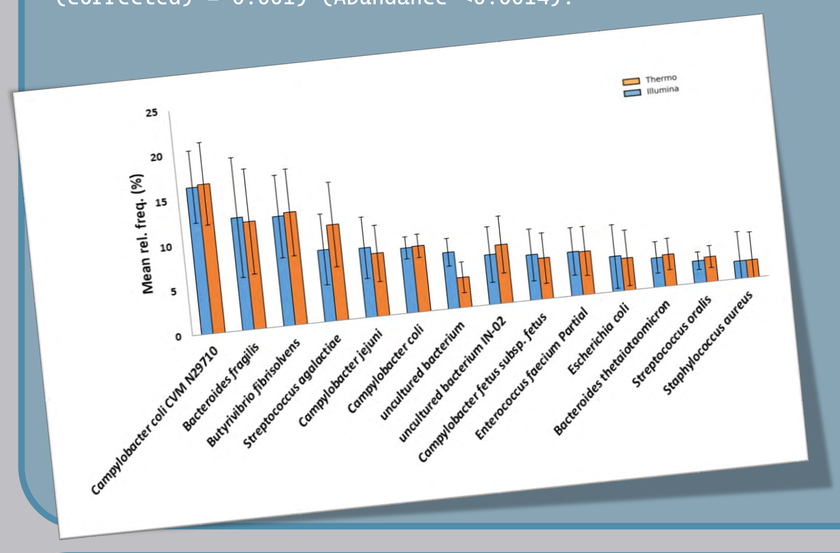


Fig. 4: The relative abundance of organisms hosting AMR genes in chicken caeca microbial populations predicted using the CARD database. Organisms represented by ≥1% of the sequence reads generated using Illumina MiSeq and Ion Torrent.

Discussion

The study was performed with 12 chicken cecum samples to estimate the abundance of AMR genes and corresponding organisms. The bioinformatics pipeline used for the data analysis with constant parameters for both platforms.

Although the initial parameters like quality score threshold and overlapping parameter vary a bit in both the platforms. Due to higher confidence at quality score greater than 30 in Illumina and greater than 20 at Ion torrent we had set different initial quality cutoffs for the data analysis. In addition to this, Ion torrent results in single-end sequencing while paired-end sequencing was used in case of Illumina.

Upon the completion of analysis, it was found that Ion Torrent resulted in higher numbers of hits (31.9%) in case of AMR detection as compare to Illumina MiSeq platform. However, those hits were found to be insignificant as their percent abundance was less than 0.004%.

The present study has effectively demonstrated that, the analysis platform used to detect AMR in samples does not significantly influence the analysis outcome. However, the selection of the method could be determined based on sample costs and availability of the resources such as instruments, consumables etc. to determine the ARGs.

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