Antimicrobial Resistance in Biofilms: Conclusions

The small number of factories, and the limited replication of meat type across factories, mean that firm conclusions cannot be drawn about the association of ARGs and biofilms in the production of different meat types. Across all samples we identified 144 ARGs, with 96 out of 144 samples containing at least one ARG. No factory has significantly higher or lower numbers of ARGs per sample than any other. However, factory B (bacon) had borderline-significantly fewer ARGs per sample than factory A (chicken). Factory D (pork and chicken) was the source of the four samples with the highest number of ARGs. The ARGs that were identified at the highest levels across samples, and in the most samples, were generally not ARGs of specific concern. Instead these tended to be regulatory genes, or biocide-resistance genes.

Using short-read DNA-sequencing of all 146 samples, we obtained a metagenome sequencing depth averaging in the high tens of millions of reads per sample, which enabled assembly into longer fragments of bacterial chromosomes and plasmids. These assemblies proved suitable for detection of DNA sequences corresponding to full-length or partial ARGs, apart from two samples for which an insufficient number of DNA sequence reads were obtained.

The long-read sequencing of a selected 21 samples enabled longer assemblies, when combined with the short-read data. This enabled some full-length bacterial genome sequences to be obtained. One case was consistent with species known to be implicated in 'kitome' contamination problems.

The qPCR assessment was mixed, with relatively few detections of the ARGs tested for. The attempts to calibrate the metagenome data using the qPCR data were also mixed, with calibration with *sul1* qPCR data being consistently higher than with 16S (albeit with only three comparable samples). However, comparison of 16S qPCR and TPM data indicates a general agreement, as follows, and the technique shows promise for further refinement.

We conducted qPCR assays for two ARGs, and also for the bacterial 16S gene generally. The results of the metagenomic sequence analysis and the qPCR were broadly in agreement. One gene, *tet*(B), being qPCR positive in only a few samples, with very low estimated copy numbers; this was negative in all samples by metagenomic ARG detection, with some evidence of a mis-assembled *tet*(B) sequence in one sample. The second gene, *sul*(I), was deemed qPCR-positive in a small number of samples, with caveats about the results for the remaining samples, which were all in low copy numbers and may have been the result of non-specific amplification. This gene was also positive in a few samples by metagenomic analysis. There were only three samples where we could directly compare the qPCR and sequencing-based results. It is difficult to draw firm conclusions but there is some indication that a few qPCR-positive samples were missed by metagenomics, due to low DNA read counts.

We also attempted to use the 16S qPCR data to normalise the relative abundance values, representing all ARGs collectively, calculated from the metagenomic data. In principle this enables direct comparison of abundances between samples. The normalisation procedure did not greatly change the total ARG abundance comparison between factories or meat-types.

The sequence data associated with this study has been deposited in the Sequence Read Archive (Accession ERP138680 / PRJEB53865).