Antimicrobial Resistance in Biofilms: Lay summary

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Antimicrobial resistance (AMR) refers to bacteria and other micro-organisms becoming resistant to the effects of antibiotics and other chemical controls. This is a globally important problem because it can stop treatments for infections working and also make medical procedures (such as chemotherapy and organ transplant) very dangerous. AMR can develop when bacteria are exposed to low levels of antibiotics and other chemicals and are given an opportunity to evolve resistance.
Bacterial biofilms are collections of sticky substances secreted by bacteria, which glue bacteria to each other and to surfaces in the environment. These protect the bacteria from the effects of chemical cleaning which can allow the bacteria to persist in food processing facilities. From an AMR perspective, biofilms might increase the risk of AMR developing, and spreading among different bacteria. This spread can occur as bacteria are sometimes able to exchange DNA which contains instructions for protecting themselves from chemicals. These instructions are referred to as Antimicrobial Resistance Genes, or ARGs.

We undertook a research project to identify the ARGs present in bacterial biofilms in meat processing plants, using techniques that allow us to generate large amounts of DNA sequence data from biofilm samples. First, we examined previously published studies to see whether any meats were particularly prone to AMR, but we didn't find sufficient evidence to make any meat a

particular focus for this study. We also used these studies to identify the locations within factories where biofilms were most likely to form, which include moist, hard to clean surfaces, and maybe those which have scratches (for the bacteria to grow in) and which are exposed to meat juices (which can help the bacteria grow).

We then developed a method for sampling bacterial biofilms from surfaces. It was important that this method was standardised, so that the samples which were taken from different factories and by different people were comparable. A total of four factories agreed to participate in this study. We used the information from previous studies, and from conversations with technical managers at the factories, to compile lists of the sites within factories where biofilms were most likely to form. We sent the sampling site lists, and the detailed sampling method to the factories, along with all the equipment needed to take samples of biofilms. The factories took the samples for us, and these were returned to our laboratory for analysis. There were 146 samples taken in total, across the four factories. Sampling took place over the course of 27 days, in summer 2021.

DNA was extracted when samples arrived at the laboratory and then analysed in three different ways. All extracts underwent short-read non-targeted DNA sequencing – this means that we took the DNA that was extracted from the biofilms and determined the DNA sequence (the order in which the As, Ts, Gs and Cs of the genetic code occurred) of lots of short fragments of DNA from the sample. We also tested 21 of the samples on another DNA sequencer that generates sequences from much longer fragments of DNA, to see if this could tell us different things about the samples. Finally following the sequencing, we tested all the samples where we had some DNA left (118 samples) using a different, targeted method called qPCR (quantitative Polymerase Chain Reaction). The aim was to try to detect three specific genes (two ARGs and a gene that is common to all bacteria). The qPCR technique gives different sorts of results to the sequencing, and can tell us how many copies of a gene there are in a sample, and therefore whether an ARG is particularly abundant in one sample compared to another. The first technique (short-read non-targeted sequencing) was used to identify the ARGs that were present, the other two techniques (long-read sequencing and qPCR) were used more experimentally to assess the suitability of these methods for future use.

We gained large numbers of DNA sequences from 144 out of the 146 samples we received (two failed sequencing). On average we generated over ninety million DNA sequences per sample. When these sequences were examined for ARGs, we found 144 ARGs (coincidently the same number as the number of samples) across all samples. Ninety six out of the 144 samples were positive for at least one ARG. One observation which stands out is that we also generated large amounts of DNA sequence from some of our negative-control samples (for example, extracts taken from unused swabs). When we look at those sequences, we can see that they belong to bacteria that are found in the samples. These bacteria, or their DNA, were probably present in the kits before they were used for sampling. This is a known phenomenon that is frequently observed. By using strict filtering of our data, we were able to remove the effects of this DNA from our results.

If we look at some of the ARGs that are found in high levels within samples, we could identify a wide range of genes that we would expect. Some of these are genes that confer resistance to antiseptics and cleaning products. Others are genes that are likely to come from bacteria that are particularly good at forming biofilms, but whose presence does not guarantee that a bacterium is actually resistant to antimicrobials. Finding these ARGs is a consequence of the database that we were using. Whilst comprehensive, it does include genes that may confer AMR only under certain conditions, genes that confer resistance only when present in conjunction with other genes, or genes whose primary functions are unrelated to AMR. Therefore, they are considered ARGs in the broadest possible sense, and predicting the ability to resist antibiotics from the presence of these ARGs is difficult.

There are very few similar studies that we can compare our data to, as these techniques are not yet widely applied. When we compare our results to those from a study of bacteria from chickens (which used an older technique) we see that our biofilms generally have lower levels of ARGs than the chicken bacteria samples. We can't tell whether this is due to real differences in the samples or is a result of the different techniques used.

Looking at the other techniques we trialled, we did see some benefit of using the long-read sequencing. We were able to identify more instances of ARGs being present on the same piece of DNA. This could be important to know, as two ARGs present on one piece of DNA may be more easily transmitted together between bacteria than two ARGs present on different pieces of DNA. The qPCR approach produced mixed results. The two ARGs that we tested were difficult to distinguish from background noise, although some of the results for one ARG did agree with the results of the sequencing. Using the qPCR data to calibrate the sequencing gave different results depending on which genes we looked at, and is therefore not yet a robust technique, but it may be a technique worth exploring in the future.
Overall, we have identified ARGs in two thirds of all the biofilm samples we looked at, across factories processing and handling the four major meat types in the UK (chicken, pork, beef, lamb). However, using this data to estimate how much these biofilms are actually contributing to ARGs in finished products would require additional sampling. Our experimental approaches showed promise for the future.