

Annotation of microbial resistance genes in household biofilm

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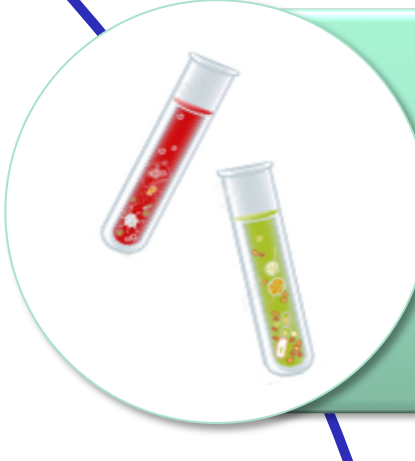
1

Intro

As a consequence of overuse and misuse of antibiotics in medicine and animal farming, antimicrobial resistance (AMR) represents an increasing problem. AMR threatens our ability to effectively treat infections and represents a serious threat to global public health. Households (and especially kitchens) have been shown to inhabit many bacteria that are resistant to antimicrobials [1,2]. Several studies have investigated the effect of antimicrobial cleaning agents on the occurrence of AMR, but finding no significant effect [3,4]. All of the studies on AMR in households have been on a phenotypical level, and thus not many studies have been made of the genotypical level. In this study, we investigate the occurrence of antimicrobial resistance genes in biofilm from household kitchen sinks and shower drains using whole metagenome sequencing.


2

Methods




Sampling & Sequencing

- Sampling biofilm from kitchen sink (32 samples) and shower drain (32 samples)
- DNA extraction → DNA fragmentation → End-repair and adenylation → Adapter ligation → PCR
- Sequencing by Illumina → Raw reads



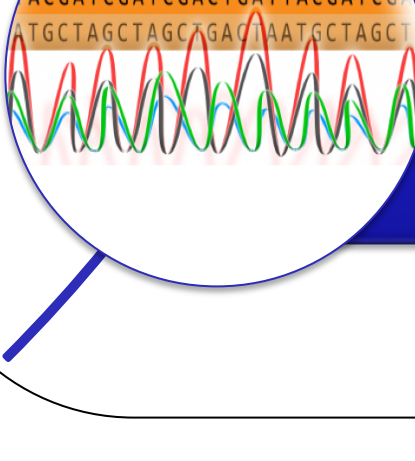
Pre Processing

- Quality control using FastQC
- Trimming of raw reads using Cutadapt
- Quality control using FastQC
- Deduplication is done to remove redundancy using SuperDeDuper



Assembly

- Assembly of the high quality paired end reads by the de novo sequence assembler, SPAdes3
- Quality check of assembly using Quast



Data analysis & visualization

- BLAST contigs against CARD resistance gene database using RGI to annotate resistance genes
- Statistical analysis and visualization in R and Python

3

Results: Resistance Gene Identifier Heatmap

The assembled contigs were mapped towards the Comprehensive Antibiotic Resistance Database (CARD). Approximately 3000 different resistance genes were found across the samples and were visualized in a heatmap (figure 1).

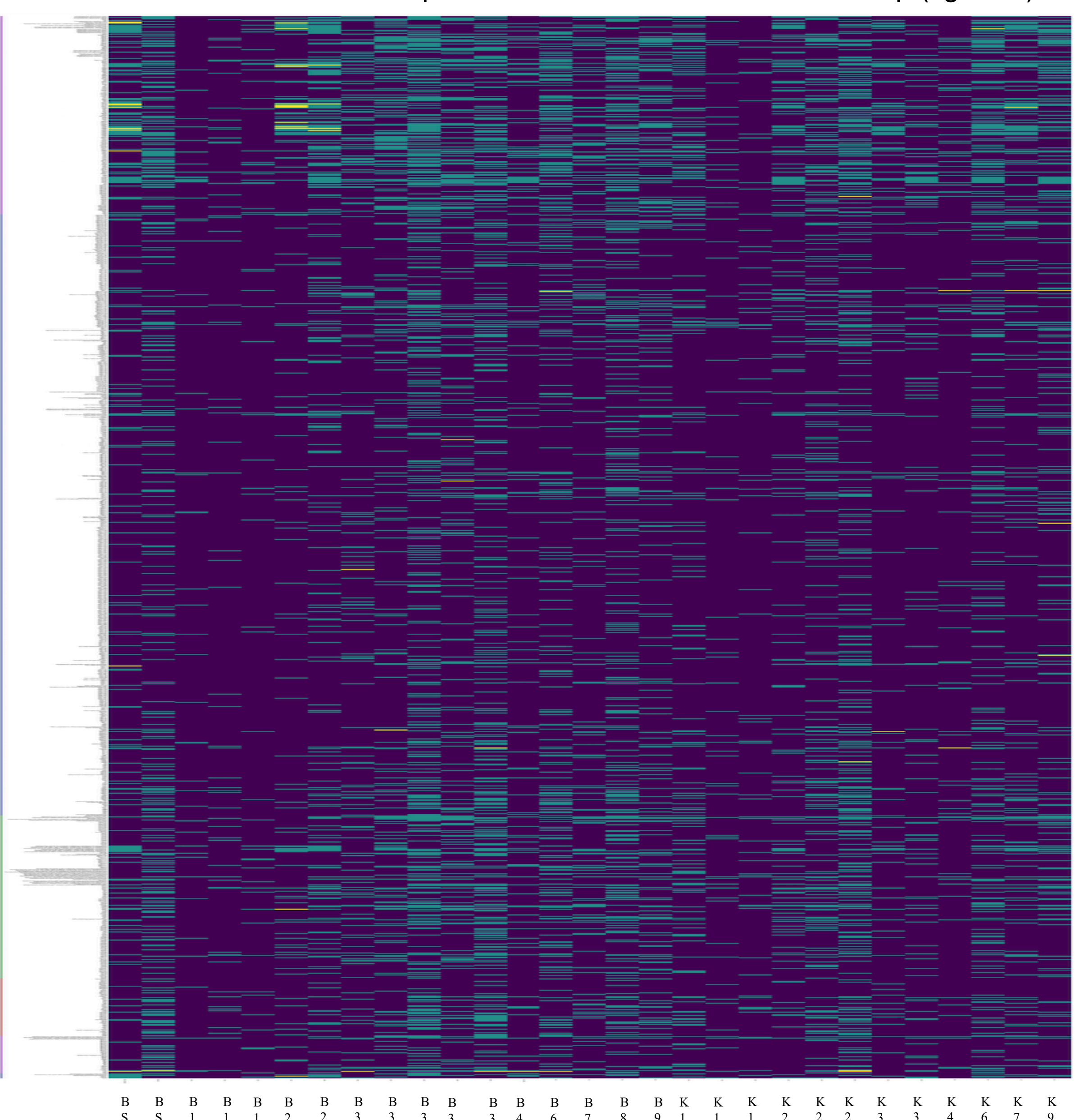


Figure 1: Heatmap over resistance genes. Yellow = Perfect (100%), Green = Strict (>95%).

4

Results and discussion

Sequencing depth cut-off

A few samples had very few annotated genes in the heatmap and a possible explanation for this could be a lack of sequencing depth. The number of genes found was plotted against the file sizes (MB) (figure 2), and it was found that a cut-off of 500MB showed to be sufficient depth. A total of 5 samples were discarded in order to prevent them from affecting further data analysis.

Distribution of antibiotics resistance genes

The distribution of drugs towards which there was found resistance, was investigated and visualized in a pie chart (figure 3). The largest groups were cephalosporin, penam and tetracycline. These findings give a good representation of the current and previous antibiotic usage in Denmark, as penicillin and tetracyclines are the most used in human treatment [6] and cephalosporin usage was discontinued in 2010 in pig production due to high levels of resistance[7].

Difference between bathroom and kitchen

Our original main hypothesis was that we would find a higher number of resistance genes in the kitchen sink, as we expected that various agricultural products might carry leftover antibiotics. In order to investigate whether there was a difference in the number of resistance genes between kitchen sinks and shower drains, boxplots of the distributions were made (figure 4). The mean of the bathrooms was slightly higher than the mean of the kitchens (630 vs. 541 genes pr. sample). However, the difference was not significant, which was confirmed by a Fisher's t-test. Additionally, no correlation between the occurrence of resistance genes and meat intake.

Effect of local water supply

It was found in the literature, that others had seen correlation between the occurrence of antibiotic resistance genes and the local water source[5], in order to determine whether there was a link between the water source of the household and the number of AMR genes in our study, the samples were divided into groups depending on the water supply: Lyngby-Taarbæk municipality (Lyngby-Taarbæk forsyning), Rudersdal municipality (Novafoss) and Copenhagen municipality (Hofo) (Figure 5). It is seen that there is a slight trend that households in Lyngby-Taarbæk municipality has a higher number of AMR genes than Rudersdal and Copenhagen municipality, however no significant correlation.

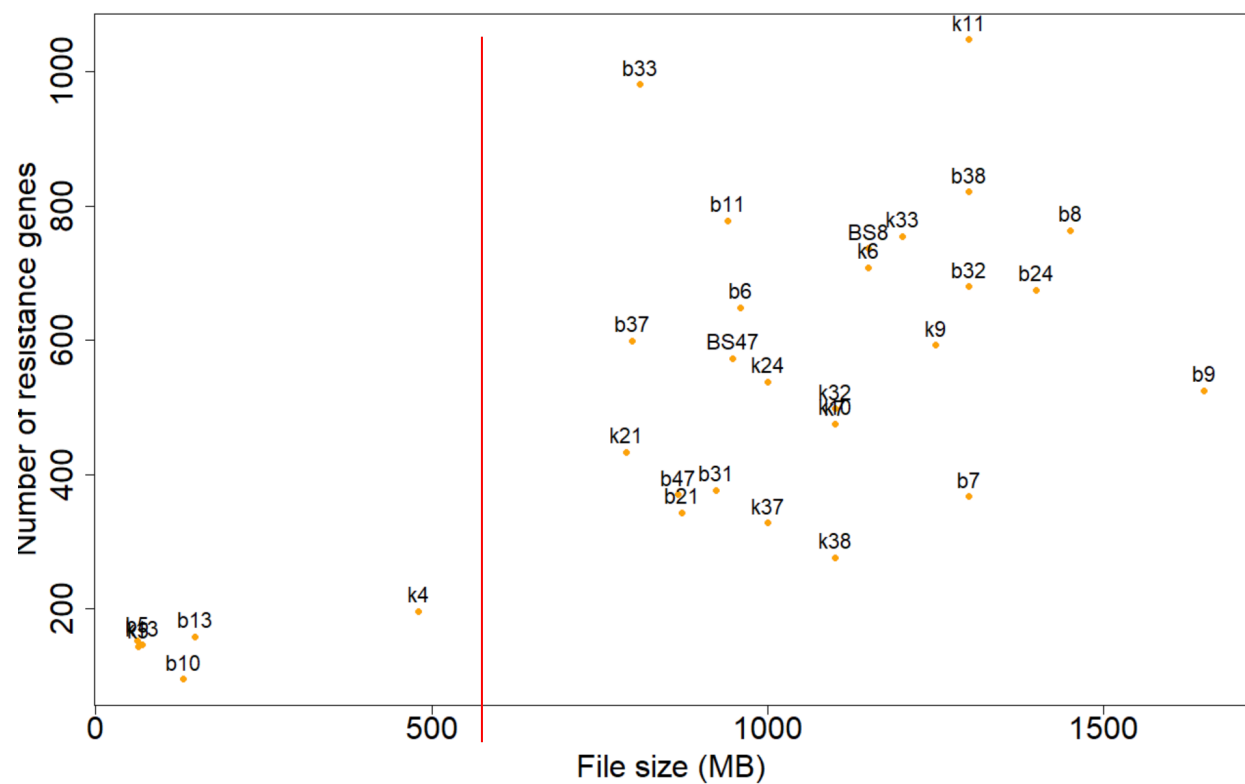


Figure 2: Number of resistance genes vs. file size. Cut-off = 500Mb

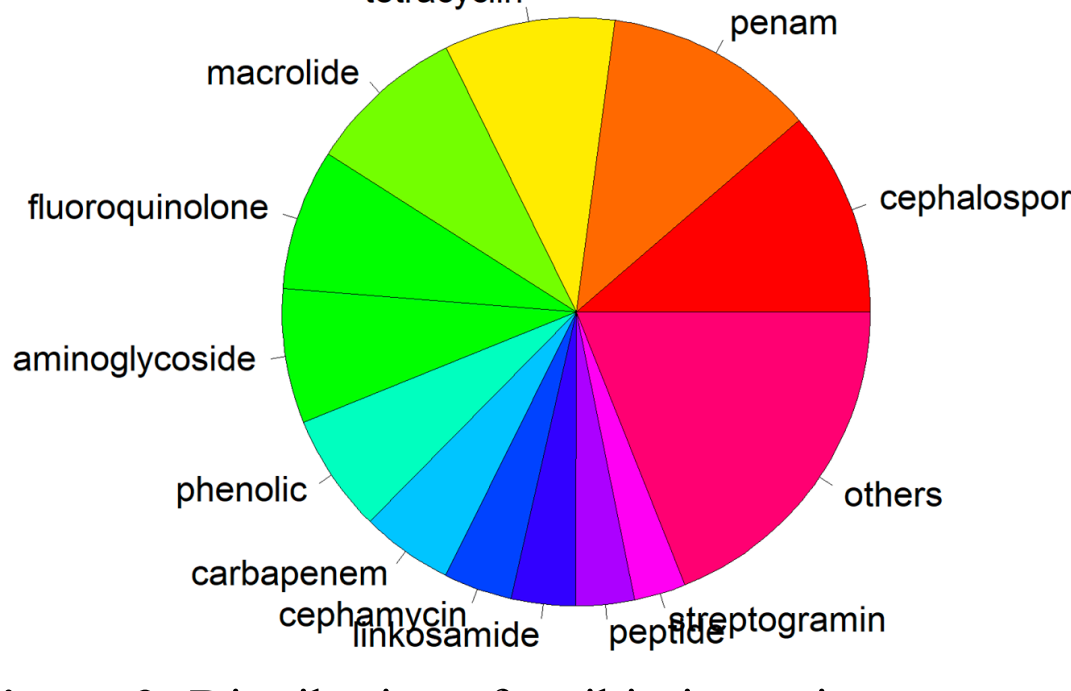


Figure 3: Distribution of antibiotics resistance genes

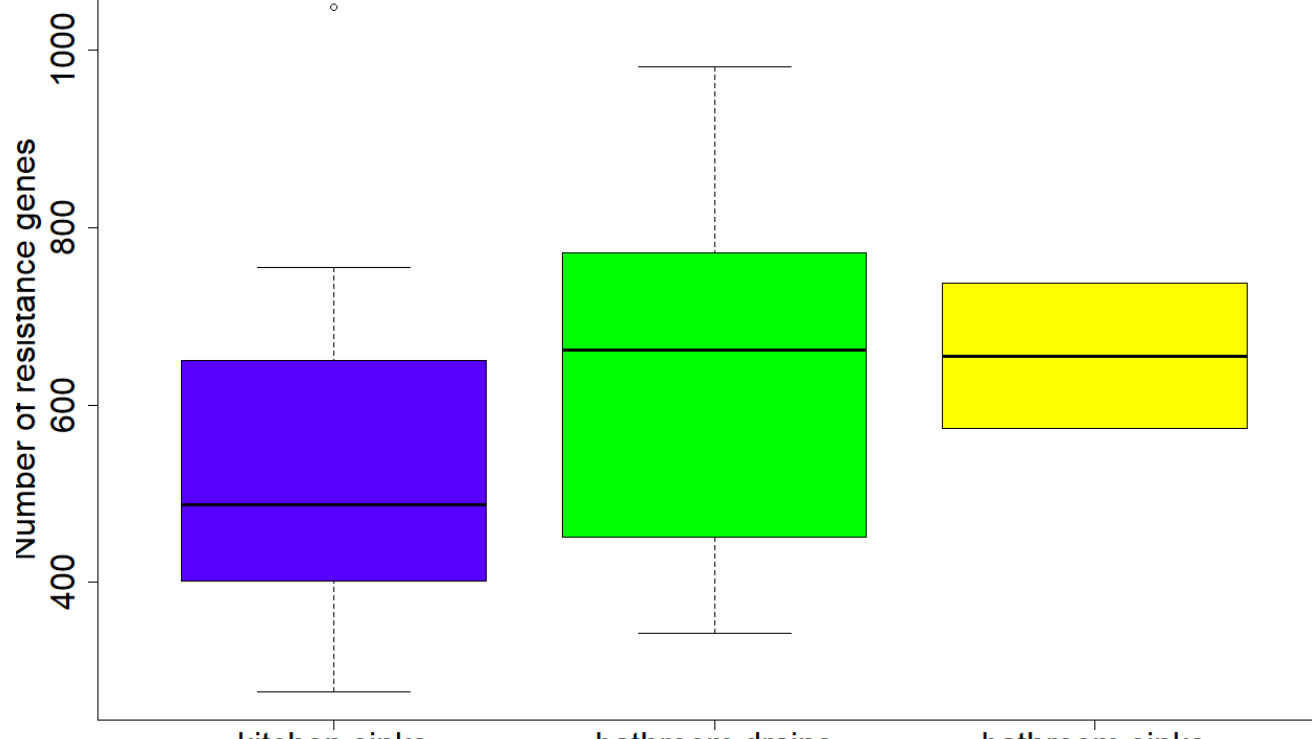


Figure 4: Boxplot of number of resistance genes in kitchen and bathroom

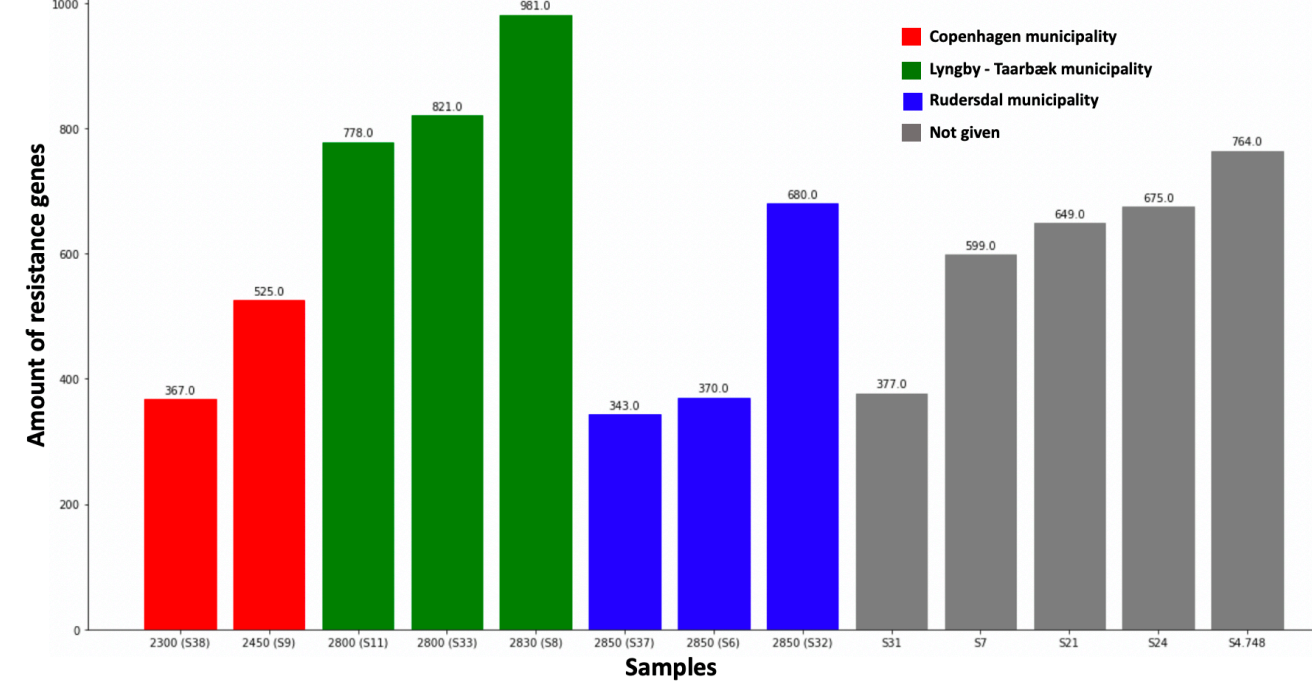


Figure 5: Diversity of resistance genes in bathroom according to water supply.

5

Conclusion

In this project, we investigated the occurrence of antimicrobial resistance genes in biofilm from kitchen sinks and shower drains using shotgun metagenomic sequencing. By mapping the assembled contigs to the CARD database, we were able to find thousands of different resistance genes. The main part of the genes were causing resistance towards cephalosporin, penam and tetracycline. The mean of the number of resistance genes was higher for bathrooms than for kitchens, however there was not a significant difference between the two. Finally, we investigated whether there was a link between postal codes and occurrence of AMR genes. There was not a significant difference between different postal codes, but there was a slight trend that households in Lyngby-Taarbæk municipality had a higher number of AMR genes than households in Copenhagen and Rudersdal municipality, however no significant correlation.

6

Further work

In metagenomic studies it is important to remember that correlation does not equal to cause, especially considering the limited sample size that we had. Thus we can not completely confirm our hypothesis that antibiotic resistance gene distribution is heavily affected by the local water source. Therefore it could be interesting to conduct a study in which samples from all different water sources in Denmark was taken, and their resistance genes subsequently annotated.

References

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