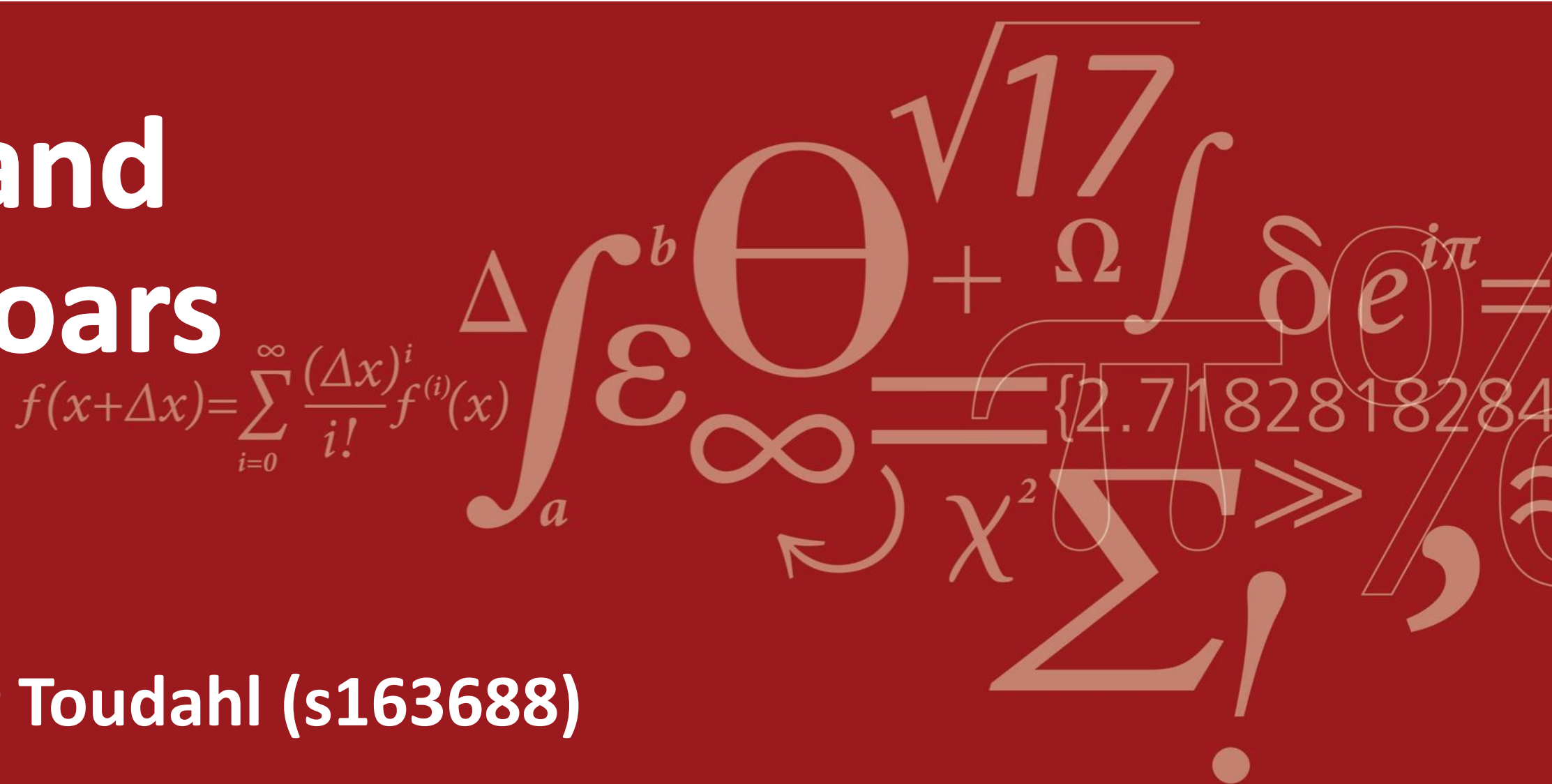# Metagenomic study of the microbiome and resistome of conventional pigs and wild boars
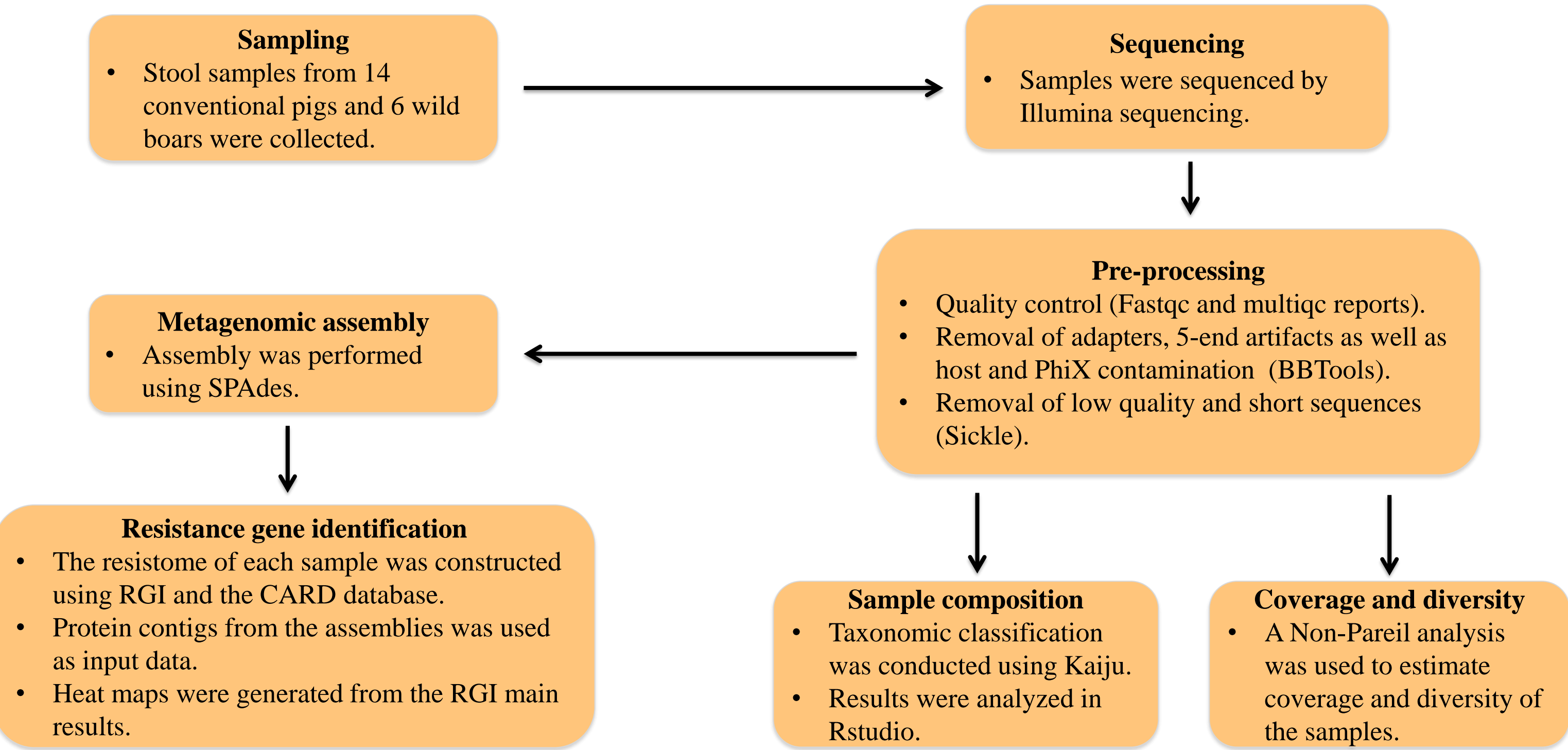
Group 5: Rasmus Christiansen (s143213), Michael Madsen (s163692), Tor Toudahl (s163688)
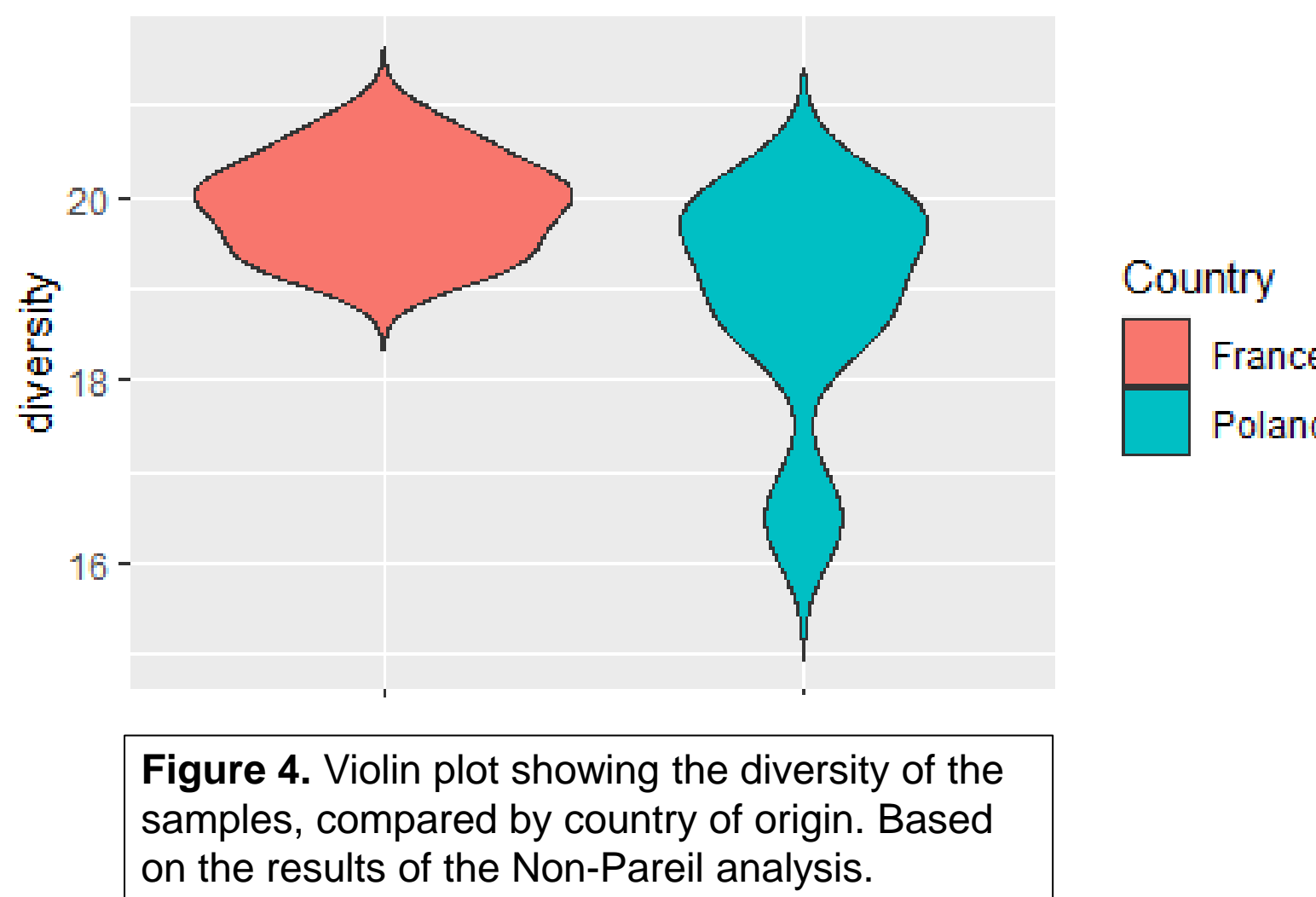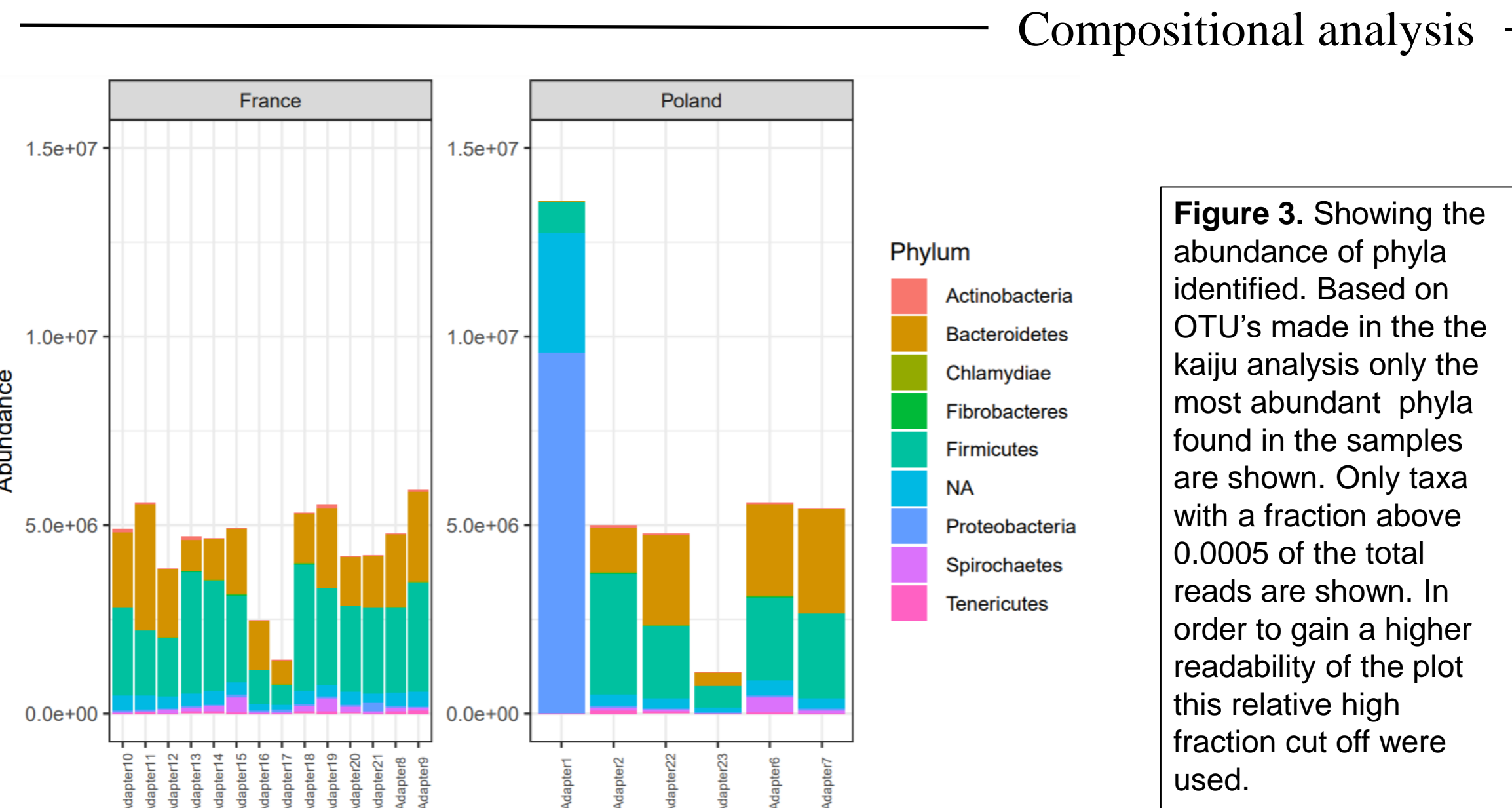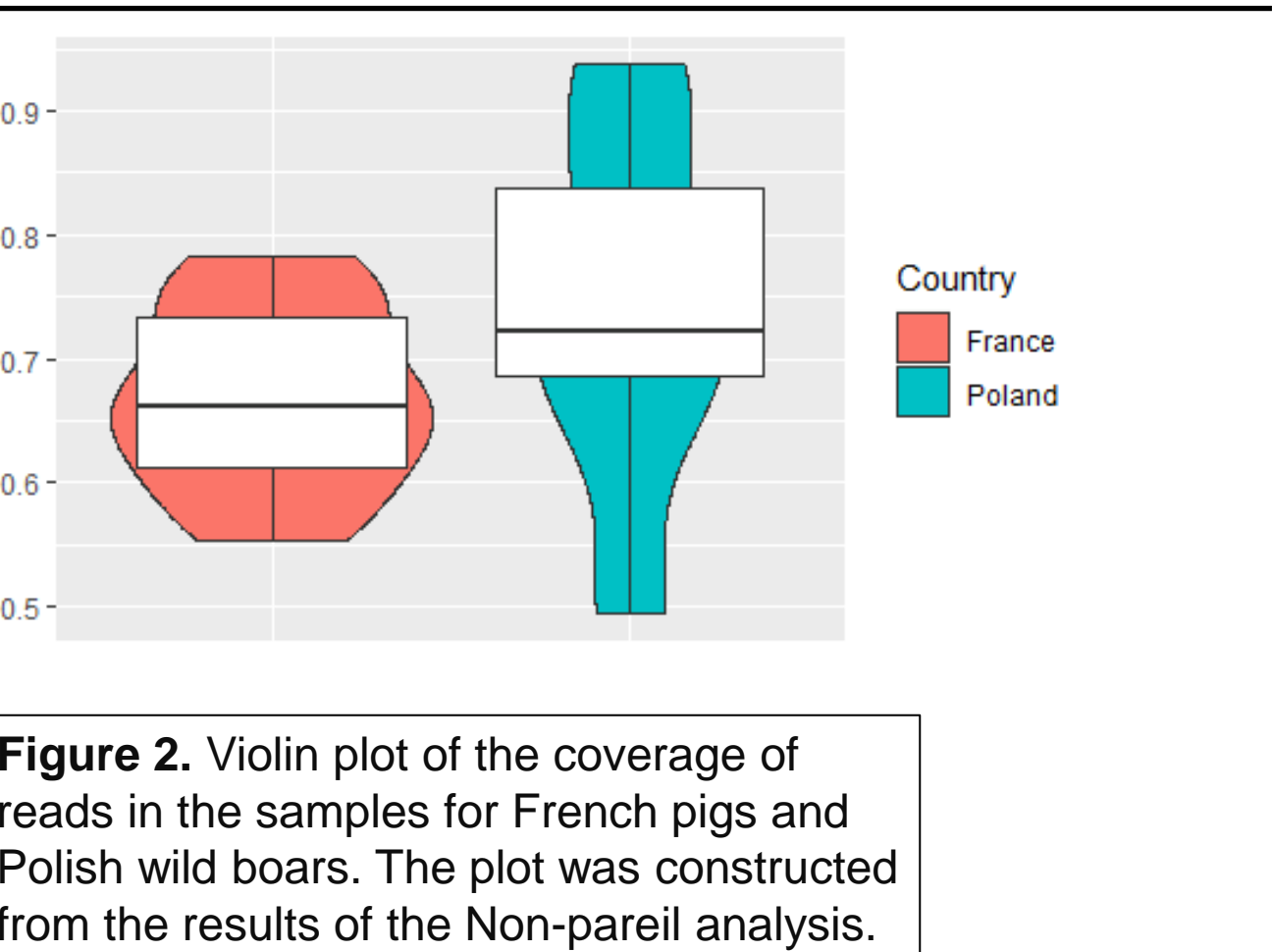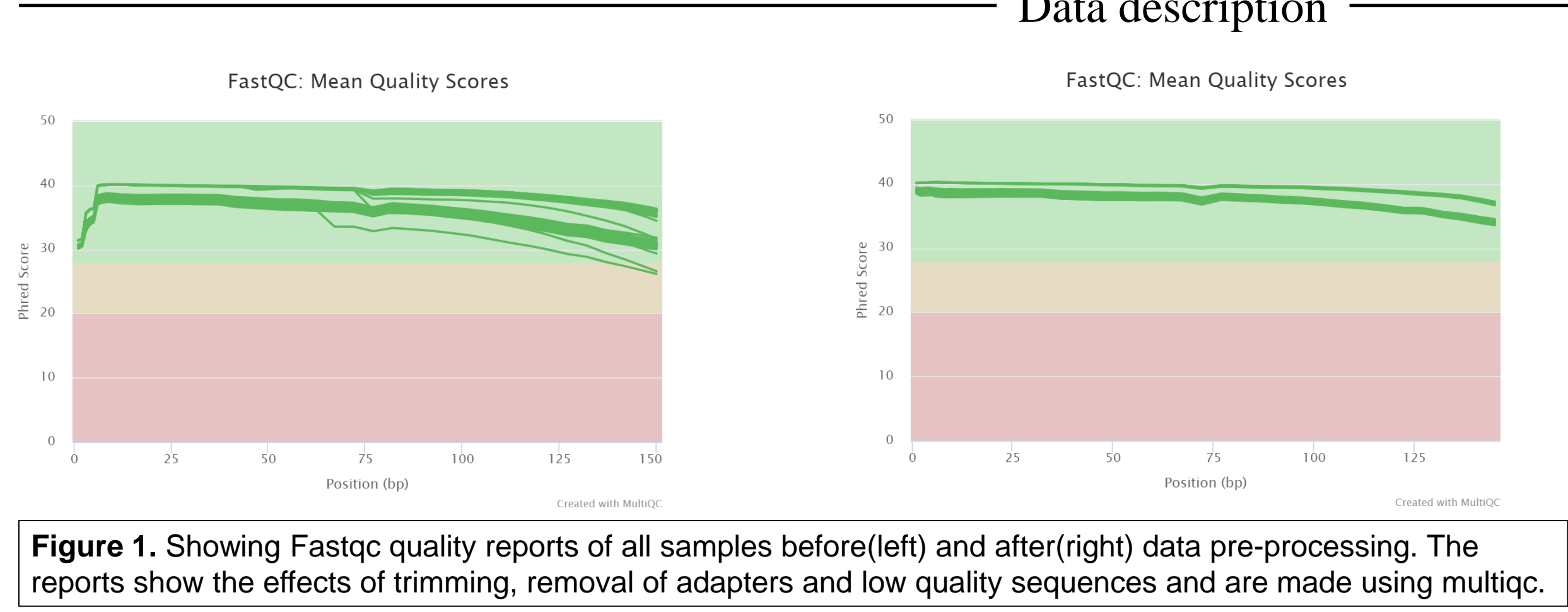
Introduction

Conventional pigs are fed with antibiotics to treat diseases [1]. In contrast, wild boars are not treated with antibiotics. The presence of antibiotics in a microbial environment causes selective pressure and facilitates growth of resistant bacteria. Twenty fecal samples of conventional pigs (14) and wild boars (6) had their metagenome sequenced and analyzed. Samples were collected as part of the EFFORT Collaboration [2]. A metagenomic analysis was used to investigate and compare the resistome and microbiome composition of conventional pigs and wild boars.

Hypothesis: The microbiome and resistome of conventional pigs and wild boars differ in composition and identified resistance genes.

Methods

Sampling
- Stool samples from 14 conventional pigs and 6 wild boars were collected.

Sequencing
- Samples were sequenced by Illumina sequencing.

Pre-processing
- Quality control (Fastqc and multiqc reports).
- Removal of adapters, 5-end artifacts as well as host and PhiX contamination (BBTools).
- Removal of low quality and short sequences (Sickle).

Metagenomic assembly
- Assembly was performed using SPAdes.

Resistance gene identification
- The resistome of each sample was constructed using RGI and the CARD database.
- Protein contigs from the assemblies was used as input data.
- Heat maps were generated from the RGI main results.

Sample composition
- Taxonomic classification was conducted using Kaiju.
- Results were analyzed in Rstudio.

Coverage and diversity
- A Non-Pareil analysis was used to estimate coverage and diversity of the samples.

Results

Data description

Figure 1. Showing Fastqc quality reports of all samples before(left) and after(right) data pre-processing. The reports show the effects of trimming, removal of adapters and low quality sequences and are made using multiqc.

Figure 2. Violin plot of the coverage of reads in the samples for French pigs and Polish wild boars. The plot was constructed from the results of the Non-pareil analysis.

Compositional analysis

Figure 3. Showing the abundance of phyla identified. Based on OTU's made in the the kaiju analysis only the most abundant phyla found in the samples are shown. Only taxa with a fraction above 0.0005 of the total reads are shown. In order to gain a higher readability of the plot this relative high fraction cut off were used.

Figure 4. Violin plot showing the diversity of the samples, compared by country of origin. Based on the results of the Non-Pareil analysis.

Resistance genes analysis

Figure 5. Heat map of both sample groups. Heat maps were generated from RGI result files using the RGI heat map functionality. The AMR genes are categorized by drug class and. A yellow bar represents a perfect hit to the CARD data base, teal represent a strict hit (>95%) and purple is no-hit. Conventional pigs (left) and wild boars (right).

Distribution of AMR drug class (Wild Boars – Poland)
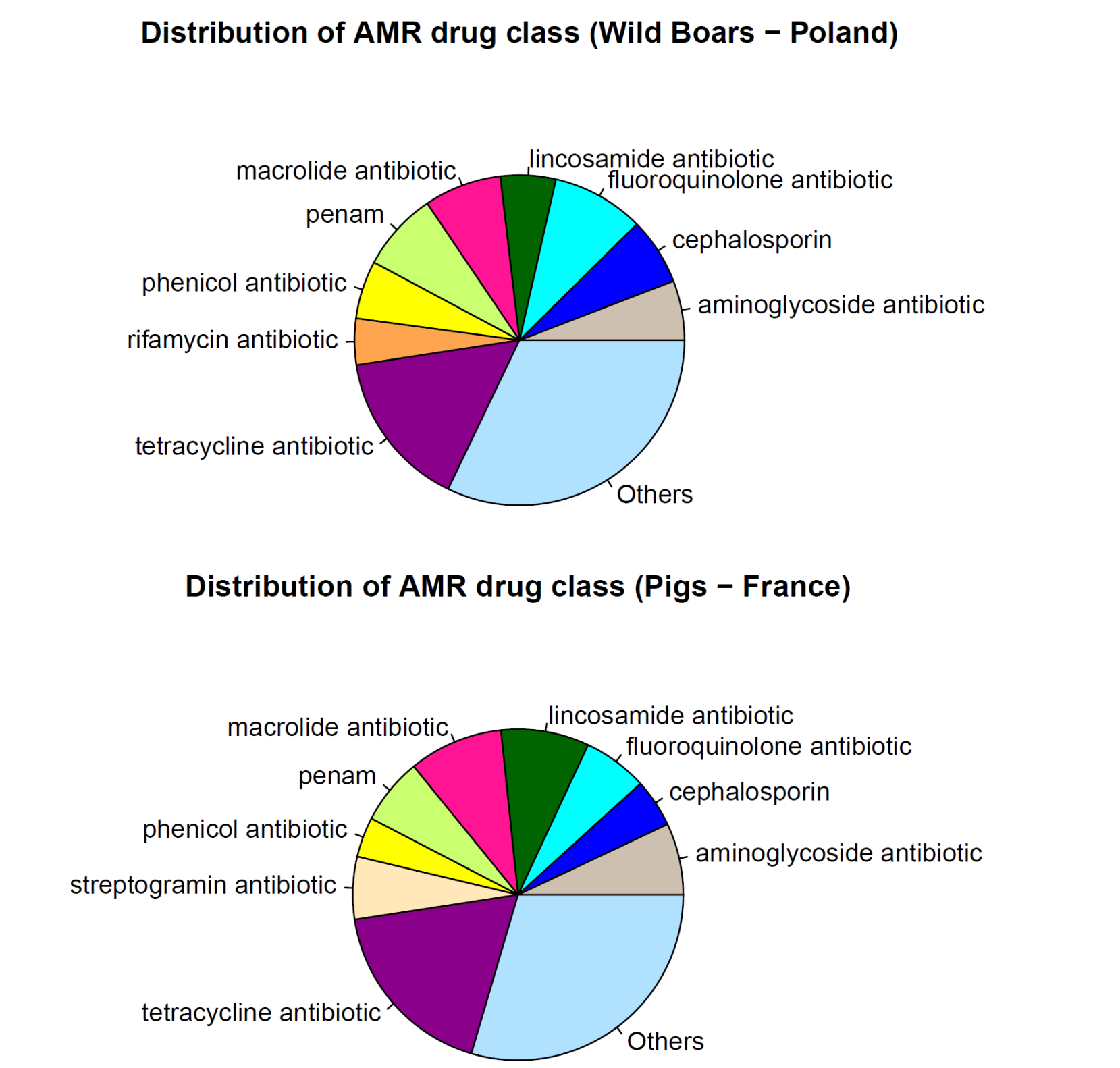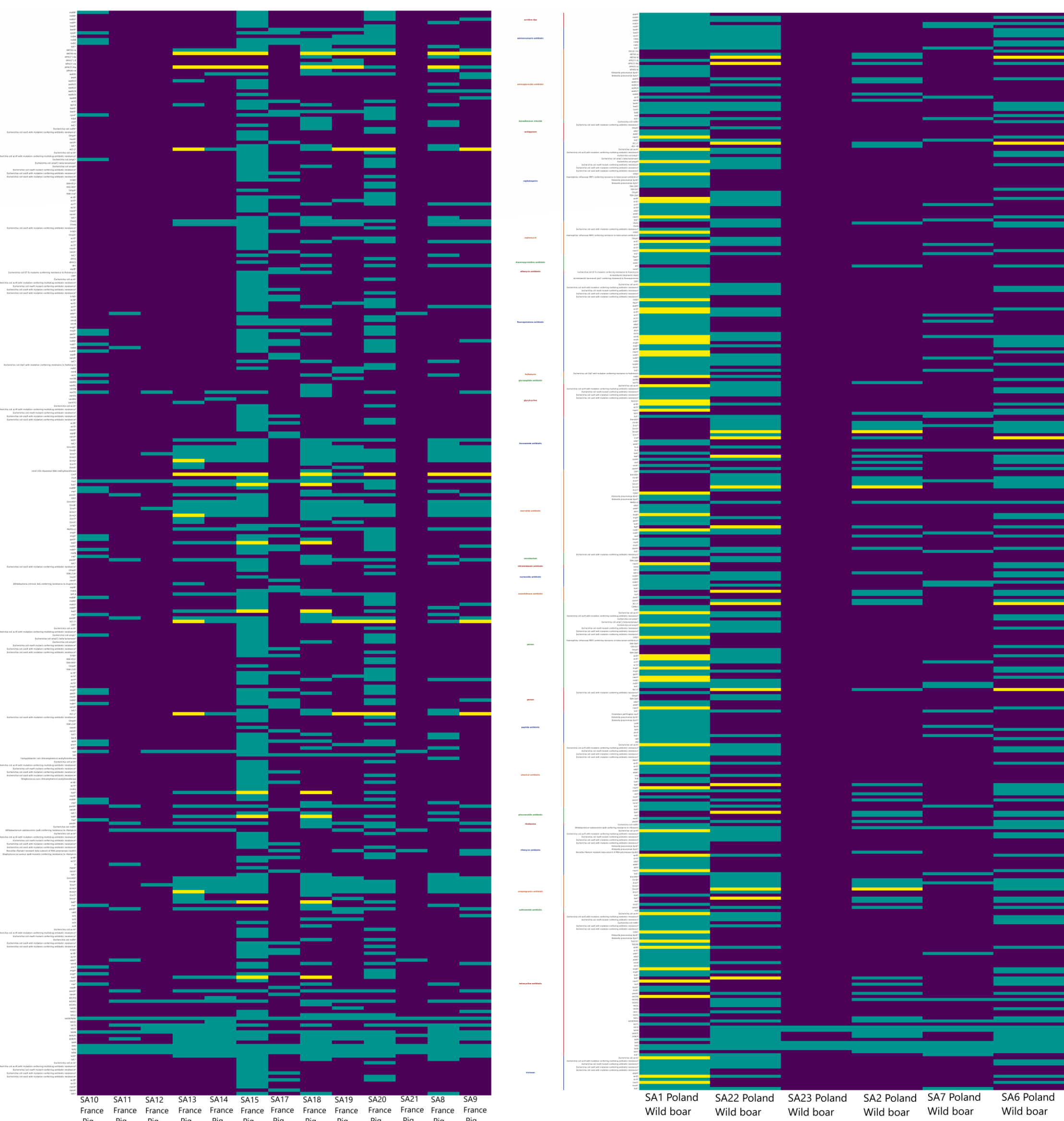
Distribution of AMR drug class (Pigs – France)

Figure 6. Showing pie charts containing the distribution of the types of antimicrobial resistance genes detected in each sample type. The 9 most commonly detected gene types are visualized and the remaining gene types are grouped in the "Others" category.

AMR	Cephalosporin	Fluoroquinolone	Glycopeptide	Penam	Phenicol	Rifamycin	Sulfonamide	Triclosan
p-value	0.0041	0.00066	0.0083	0.017	0.0048	0.016	0.045	0.010

Table 1. A Welch's t-test was performed with a significance level of 0.05 to determine significant differences in AMR genes between the two groups. The table contains significantly different AMR genes.

Discussion

In this study, resistome and microbiome composition of fecal samples from conventional pigs and wild boars were investigated.

The data pre-processing performed improved the overall quality of the reads.

A Non-Pareil analysis was performed to evaluate the coverage of the samples. As shown in fig. 2 samples within the group of wild boars have a higher average coverage (73%) compared to the conventional pig samples (66%). Furthermore, the coverage of the conventional pig samples are more widespread. Overall both groups have a high coverage indicating that the sequencing depth was adequate to capture most of the metagenome.

A Kaiju analysis was performed for taxonomic classification of each microbiome. The results of this analysis was visualized by plotting the phylum distribution of the bacteria in each sample (fig. 3). As shown in fig. 3 there was no apparent difference between the phylum distribution in the two groups. The microbiome composition of one of the wild boar samples deviates much from the remaining samples. This sample might originate from a sick individual as it has a high frequency of Proteobacteria [3].

As shown in fig. 4 the conventional pig samples have a higher diversity than those from wild boars. Comparing fig. 2 and fig. 4 reveals an apparent negative correlation between diversity and coverage. This is expected as more diversity will decrease the possibility of capturing the same reads.

Heat mapping was used to visualize detected resistance genes within the samples (fig. 5). As shown in fig. 5 there is no obvious difference between the two groups, although individual samples within each group are substantially different. The variability between the individual samples indicates that each animal has a characteristic resistome.

One wild boar sample in the heat map is clearly distinguishable from the other samples as it contains a high amount of resistance genes detected. Interestingly, this is the same wild boar sample that had a high abundance of Proteobacteria in fig. 3.

As seen in fig. 6 the types of antimicrobial resistance genes commonly detected in the two groups was similar. Although some AMR drug classes are significantly different (see tbl. 1) this might be a result of the small sample size and possibly sick individual. It is not certain that these differences would be found if a larger sample size was used.

In this study, the presence of resistance genes in the samples was investigated, but the abundance of these genes has not been elucidated. Although the two sample groups contain similar resistance gene types they might still differ in the abundance of these genes. Therefore, a study including larger sample sizes as well as an investigation of the resistance gene abundance would improve the capabilities to determine potential differences between sample groups.

References

[1] Joyce, Aoife et al. (2019) Antibiotic resistomes of healthy pig faecal metagenomes, Nature microbiology, 2018 vol. 3, issue 8.

[2] Munk, Patrick et al. (2018), Abundance and diversity of the fecal resistome in slaughter pigs and broilers in nine European countries.

[3] Sun, Jing et al. (2019), Identification of the core bacteria in rectums of diarrheic and non-diarrheic piglet, Sci rep 9, 18675