



Comparative Genomics

Preliminary Results

Team II: Fosfomicin Heteroresistance

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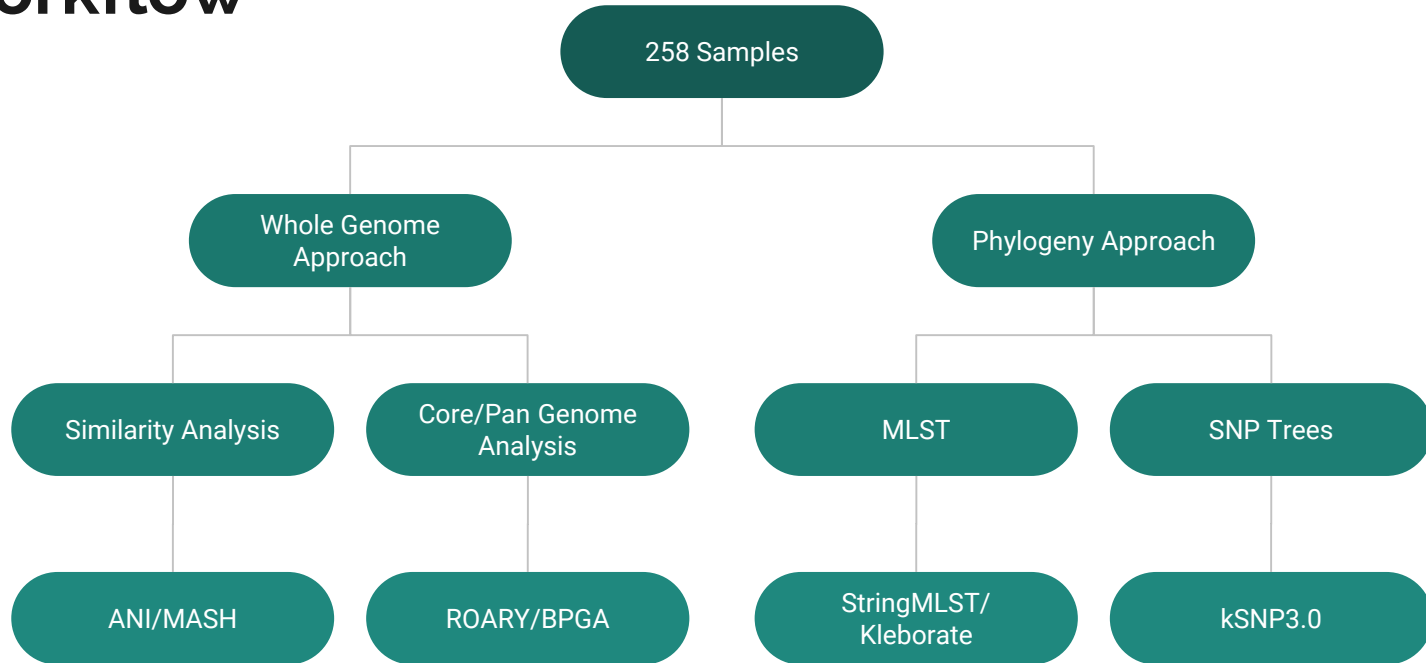
Objective

To identify genetic determinants that could be a potential cause for Fosfomycin heteroresistance in the isolates provided.

Resistant	27 (10%)
Heteroresistant	176 (68%)
Susceptible	9 (3%)
N/A	46 (18%)
Total	258



Workflow





Whole-Genome Based Methods

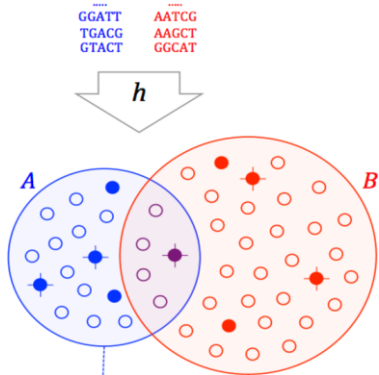
Preliminary Results

Similarity Analysis

Distance Computation

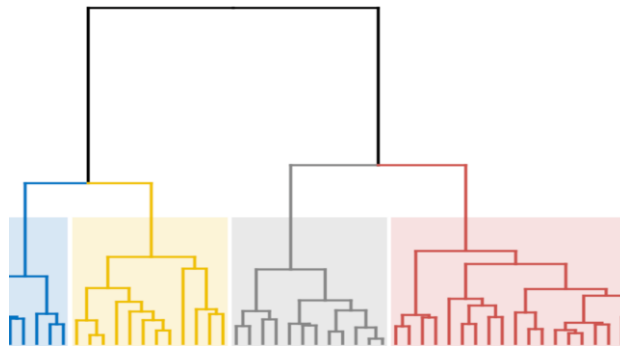
- MASH
- Jaccard Similarity Coefficient

$$J(A, B) = \frac{|A \cap B|}{|A \cup B|}$$

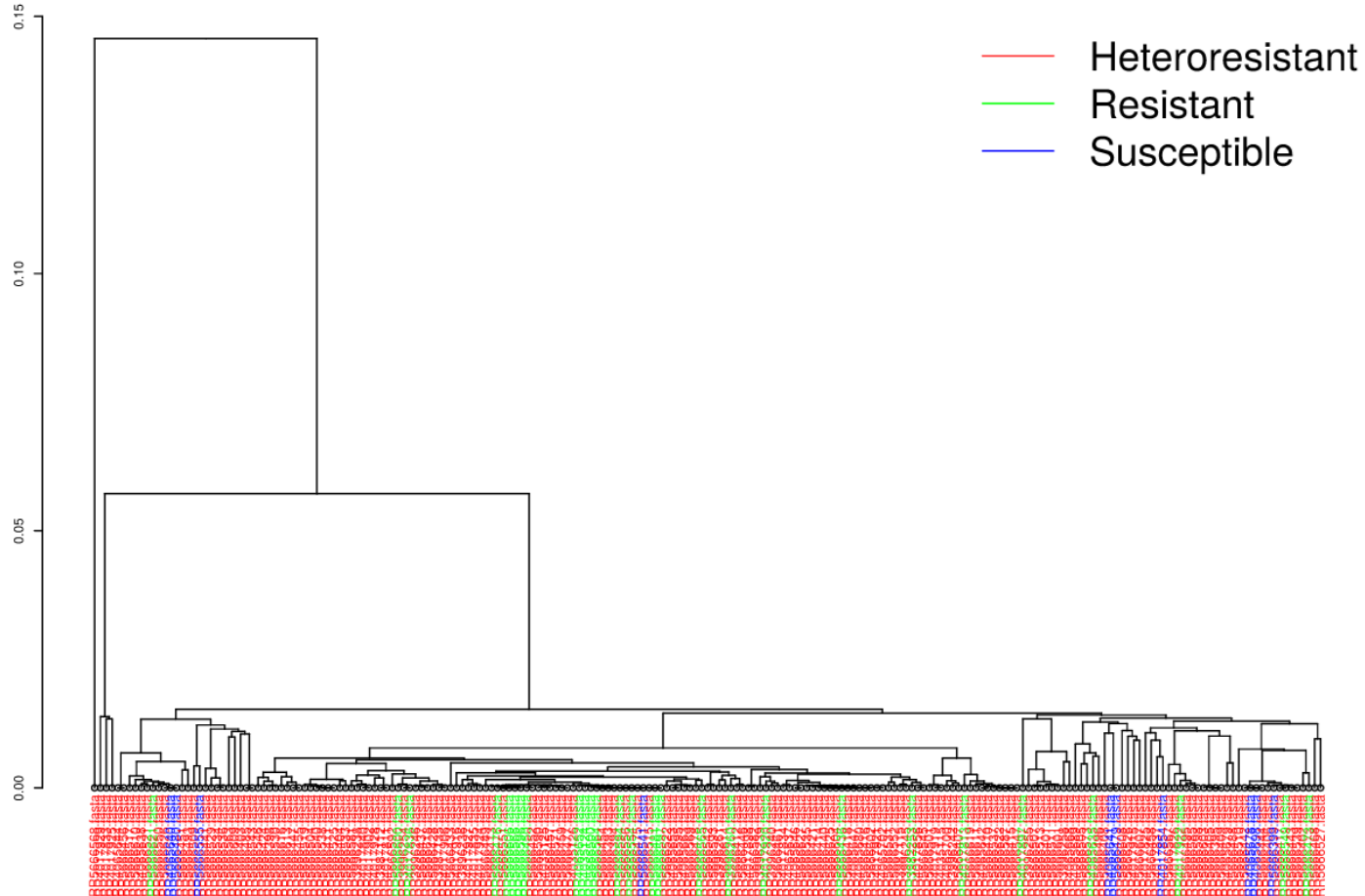


Clustering

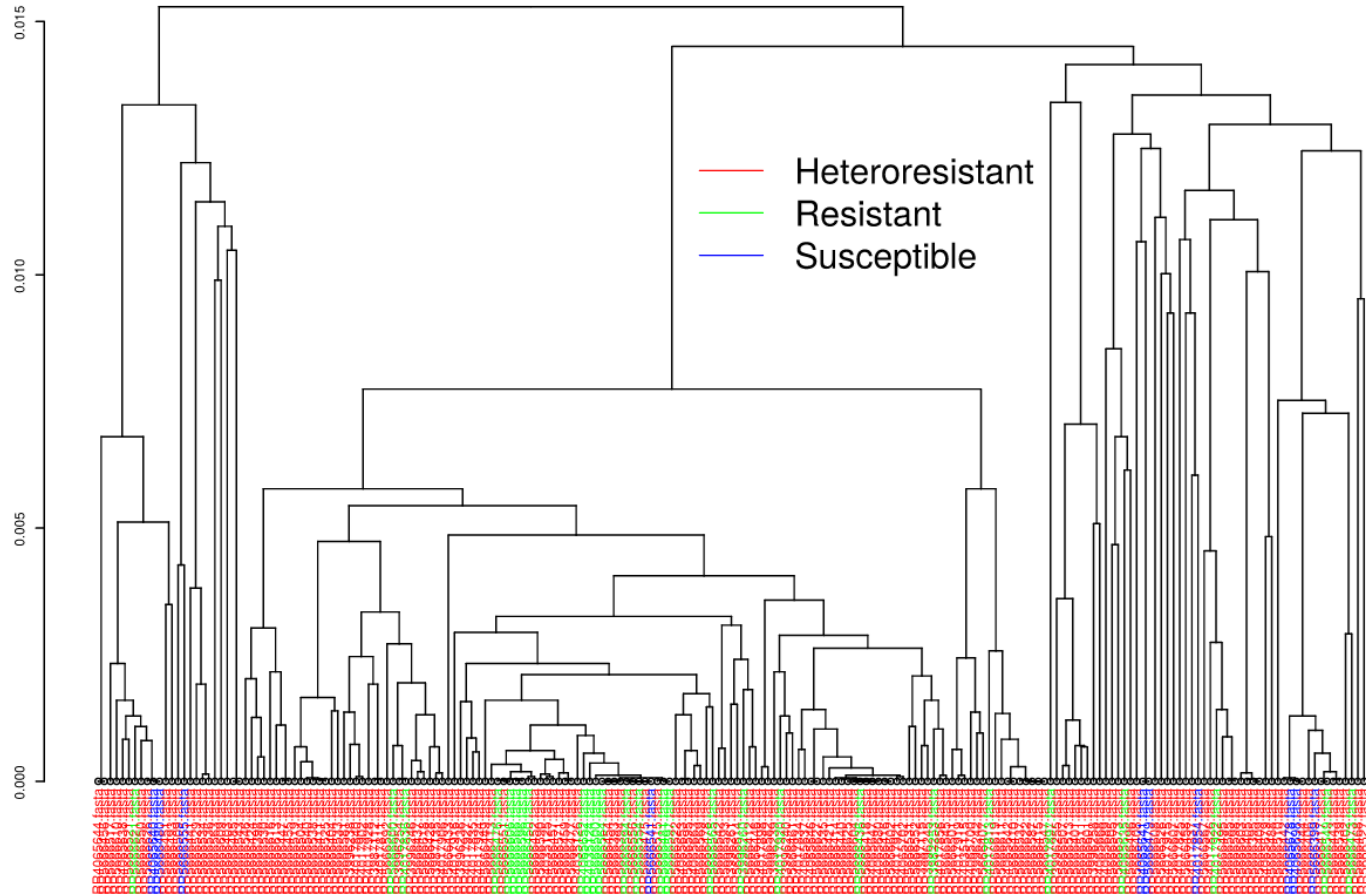
- hclust R package
- Hierarchical Clustering (Complete Linkage)
- Dendrogram



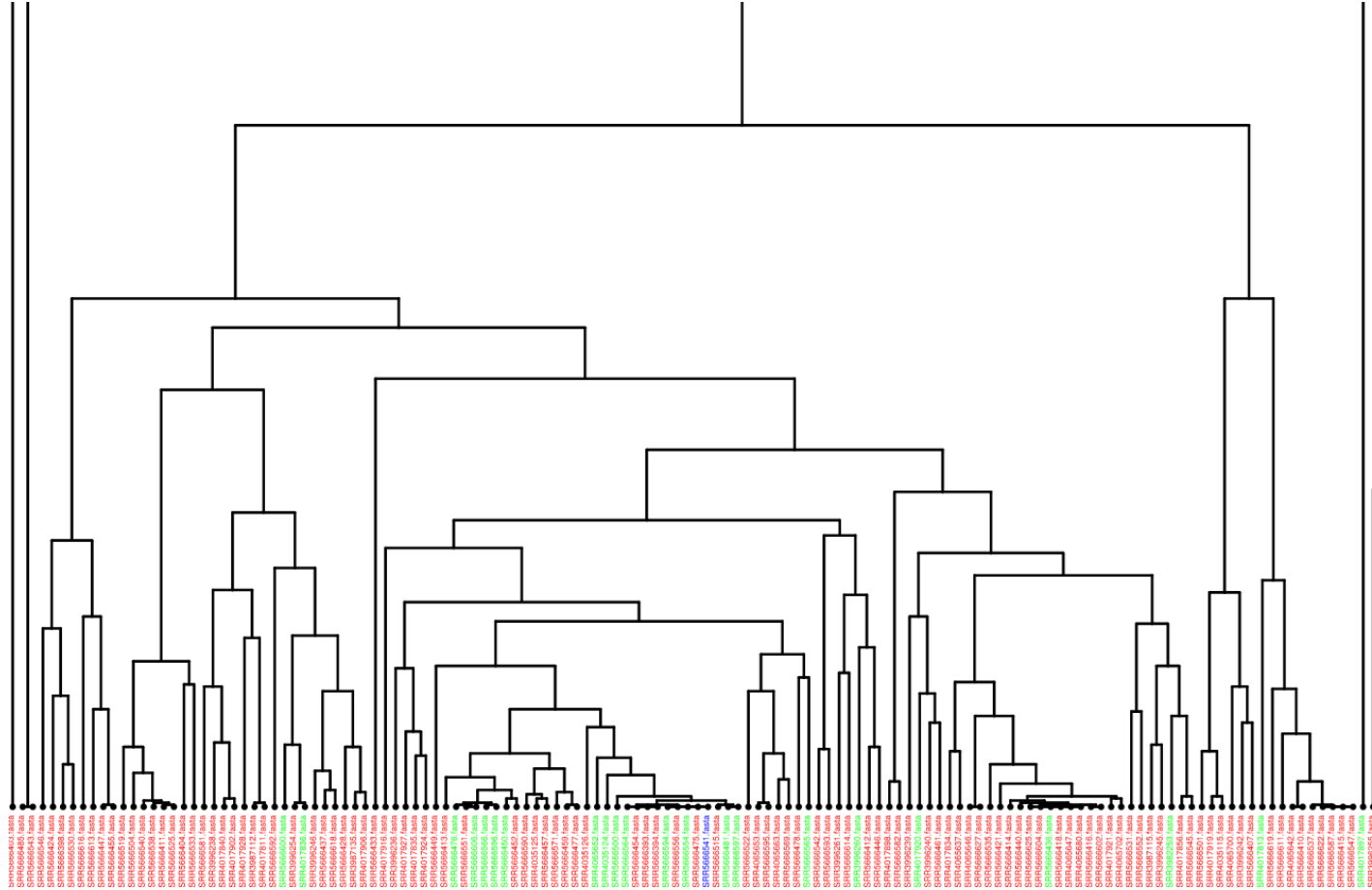
Hierarchical Clustering - Resistant Groups



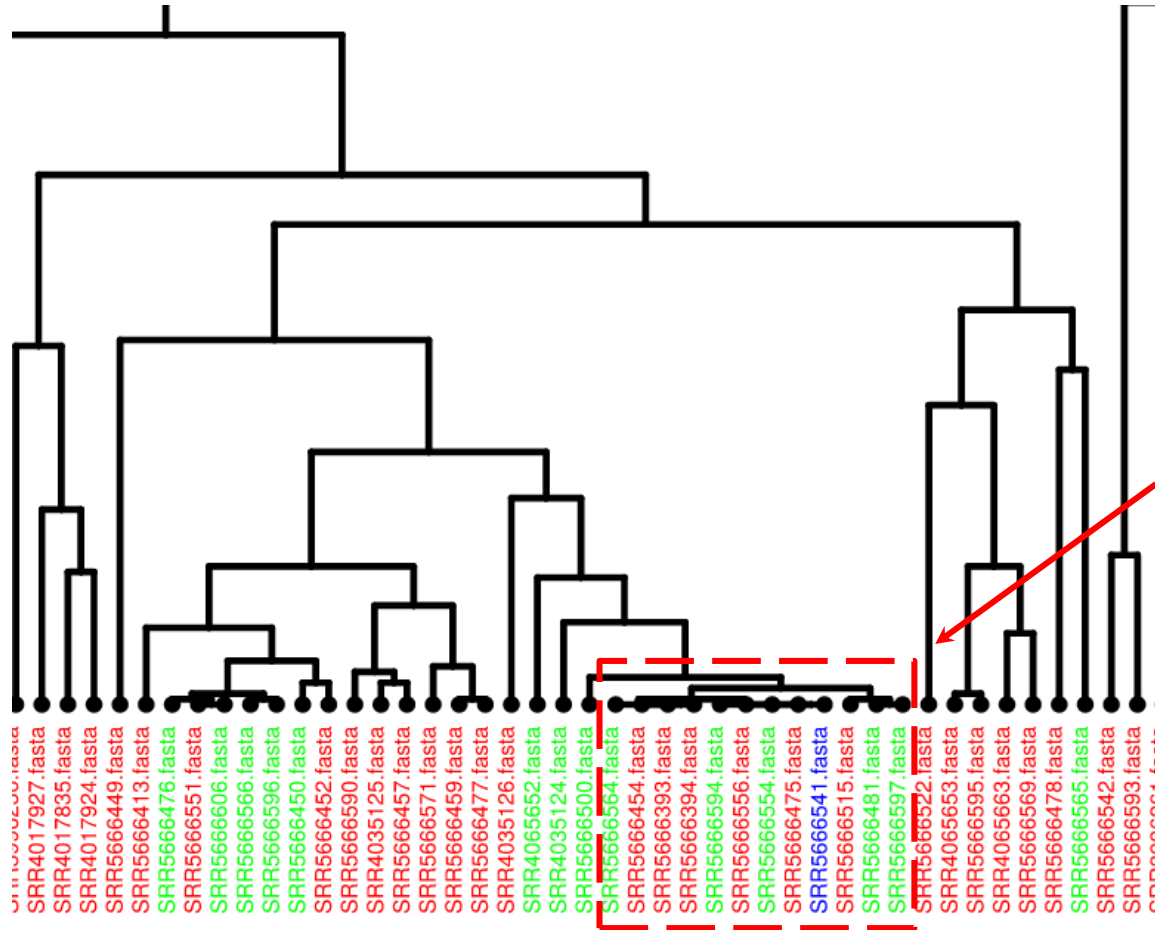
Hierarchical Clustering - Outliers Removed



Hierarchical Clustering - Zooming In



Hierarchical Clustering - Zooming In



- Subgroup of extremely similar genomes containing all resistance groups
- All differences between those genomes are worth exploring



Phylogeny Based Methods

Preliminary Results



MLST



- Kleborate - a tool for characterising virulence and resistance in *Klebsiella*
 - MLST sequence type
 - species (e.g. *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, etc.)
 - virulence genes: *ybt*, *clb*, *iro* and *iuc* loci, and the *rmpA* and *rmpA2* hypermucoidy genes
 - antimicrobial resistance genes, including quinolone resistance SNPs and colistin resistance truncations
- *[publication still under review]*
 - <https://github.com/katholt/Kleborate#mlst>



Kleborate Results

Antibiotic Resistance Genes -

It screens for resistance genes against the ARG-Annot database of acquired resistance genes

Screened for genes conferring Fosfomycin resistance for all samples

None of the samples reported any particular variant for the Fcyn gene

This is expected as the cause for hetero-resistance is not a mere mutation in genes conferring antibiotic resistance



Kleborate Results

Imprecise MLST

- Imprecise ST calls are indicated with `-nLV`, where `n` indicates the number of loci that disagree with the ST reported. So `258-1LV` indicates a single-locus variant (SLV) of ST258, i.e. 6/7 loci match ST258.

Conclusion

Since this tool is still under review - validation of results is still needed. We only extracted the Sequence typing results

MLST



StringMLST

Ran on a subset

11 samples from each group [since total susceptible samples are 11]

Compared the sequence typing results from StringMLST to those from Kleborate

- 1) For validation of Kleborate results
- 2) To avoid potentially having to download fastq files for 258 genomes - input for StringMLST

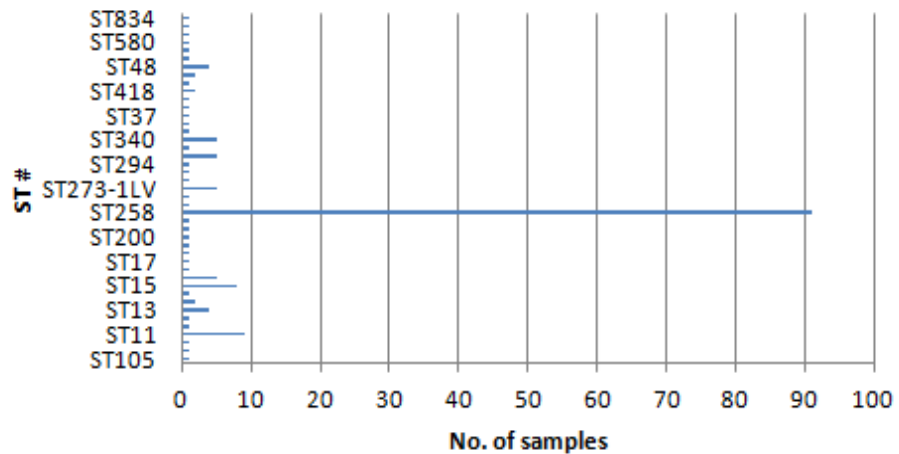
Result

Both tools reported the same ST for all the tested samples

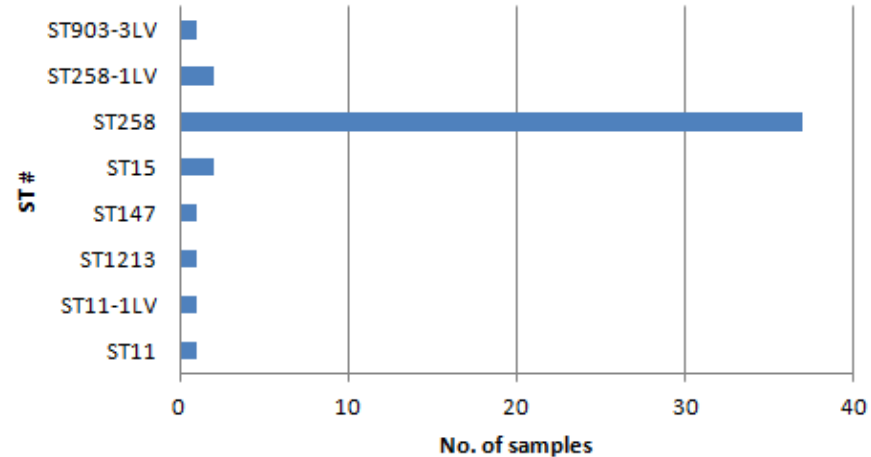
MLST - Results



Hetero-resistant



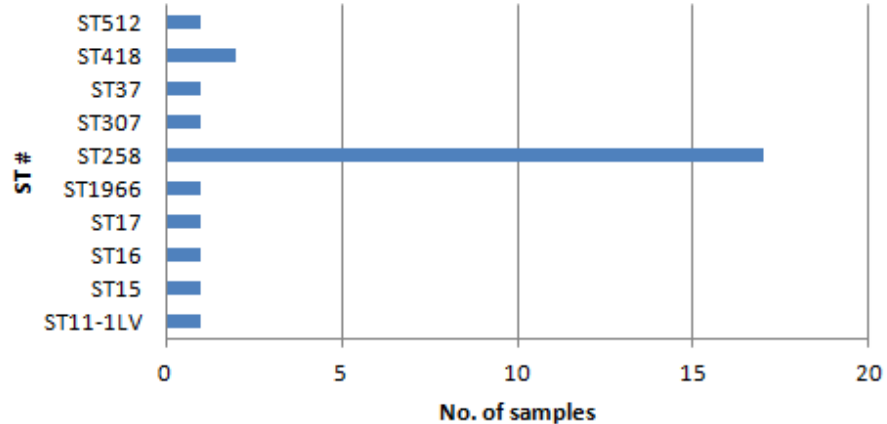
N/A



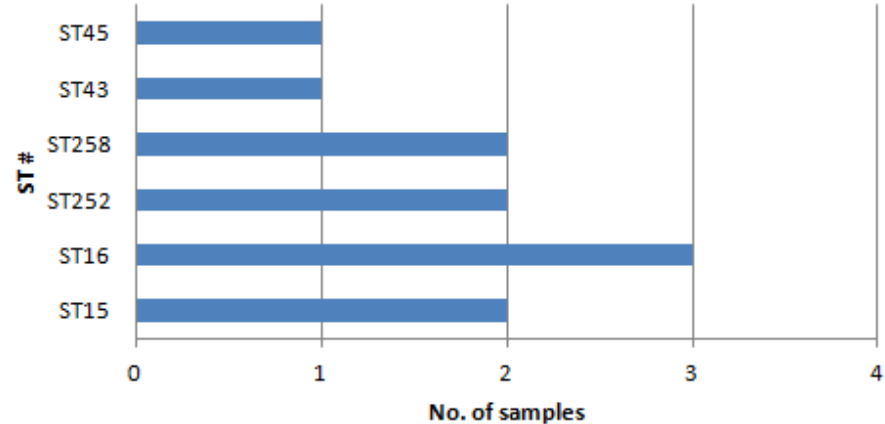
MLST - Results



Resistant



Susceptible



MLST - Results



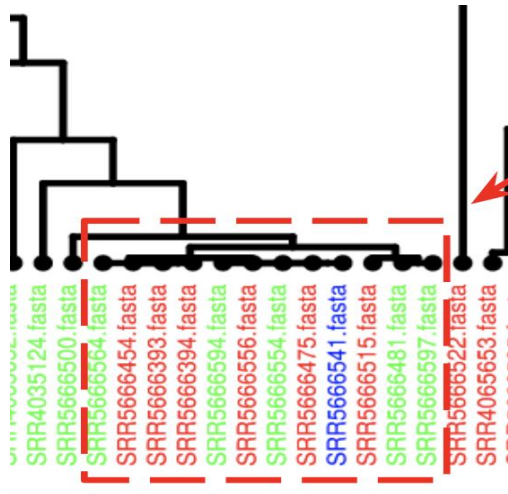
Occurrence of Sequence Types common across all the groups

ST #	Hetero	Sus	Resist	N/A
ST15	8	2	1	2
ST258	91	2	17	37

MLST - Results



Cluster of Interest



Sample Name	Phenotype	ST #
SRR5666393	Hetero	ST258
SRR5666394	Hetero	ST258
SRR5666454	Hetero	ST258
SRR5666475	Hetero	ST258
SRR5666515	Hetero	ST258
SRR5666556	Hetero	ST258
SRR5666481	Resist	ST258
SRR5666554	Resist	ST258
SRR5666594	Resist	ST258
SRR5666597	Resist	ST258
SRR5666522	Resist	ST258
SRR4065653	Resist	ST258
SRR5666541	Suscept	ST258
SRR5666541	Suscept	ST258

MLST - Conclusion



MLST using a regular HouseKeeping gene typing scheme did not return results that can help us differentiate between the phenotypes

- We need to change our typing scheme (use cgMLST/wgMLST)

OR/AND

- Start looking at SNPs

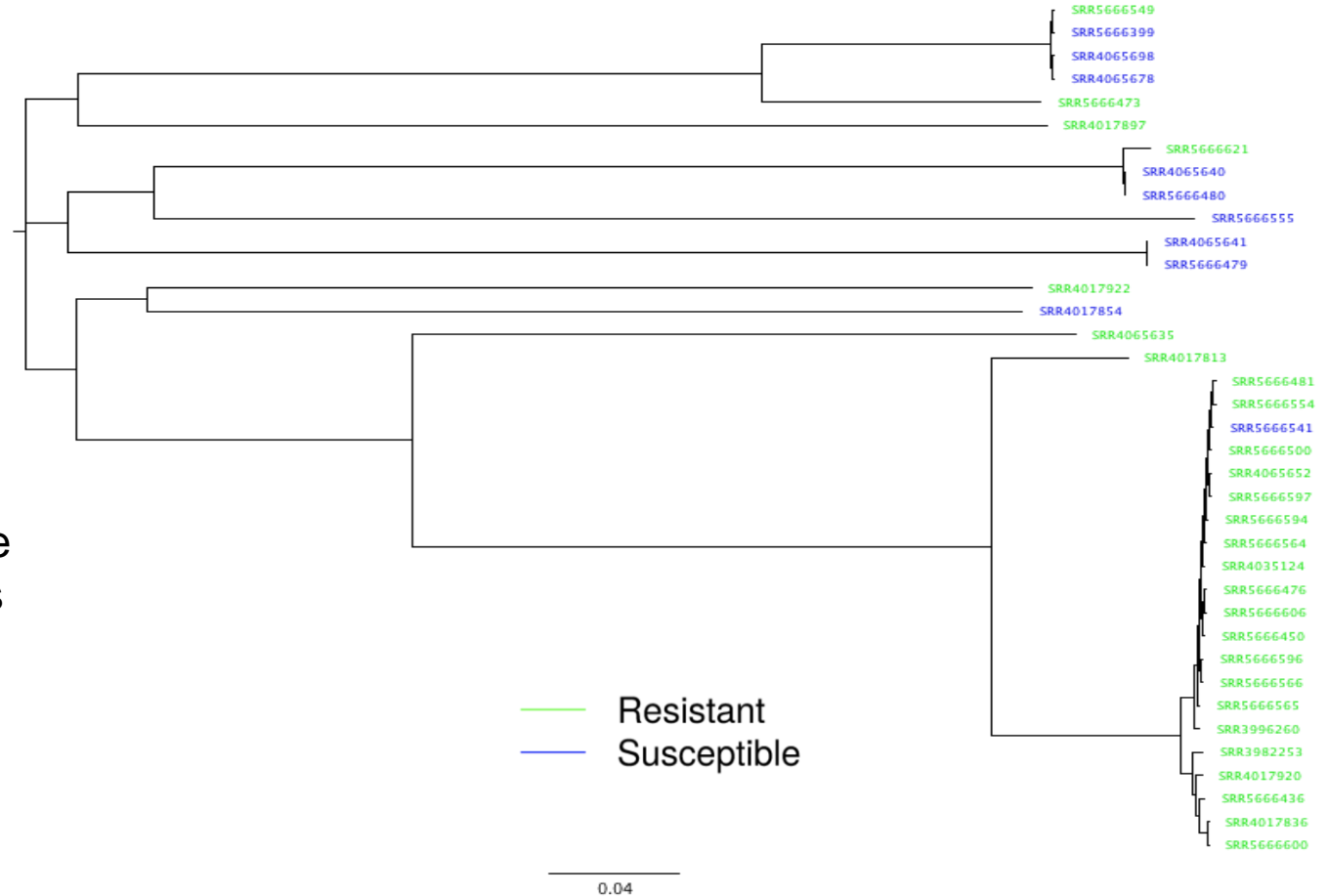


kSNP3.0

SNP identification and phylogenetic analysis

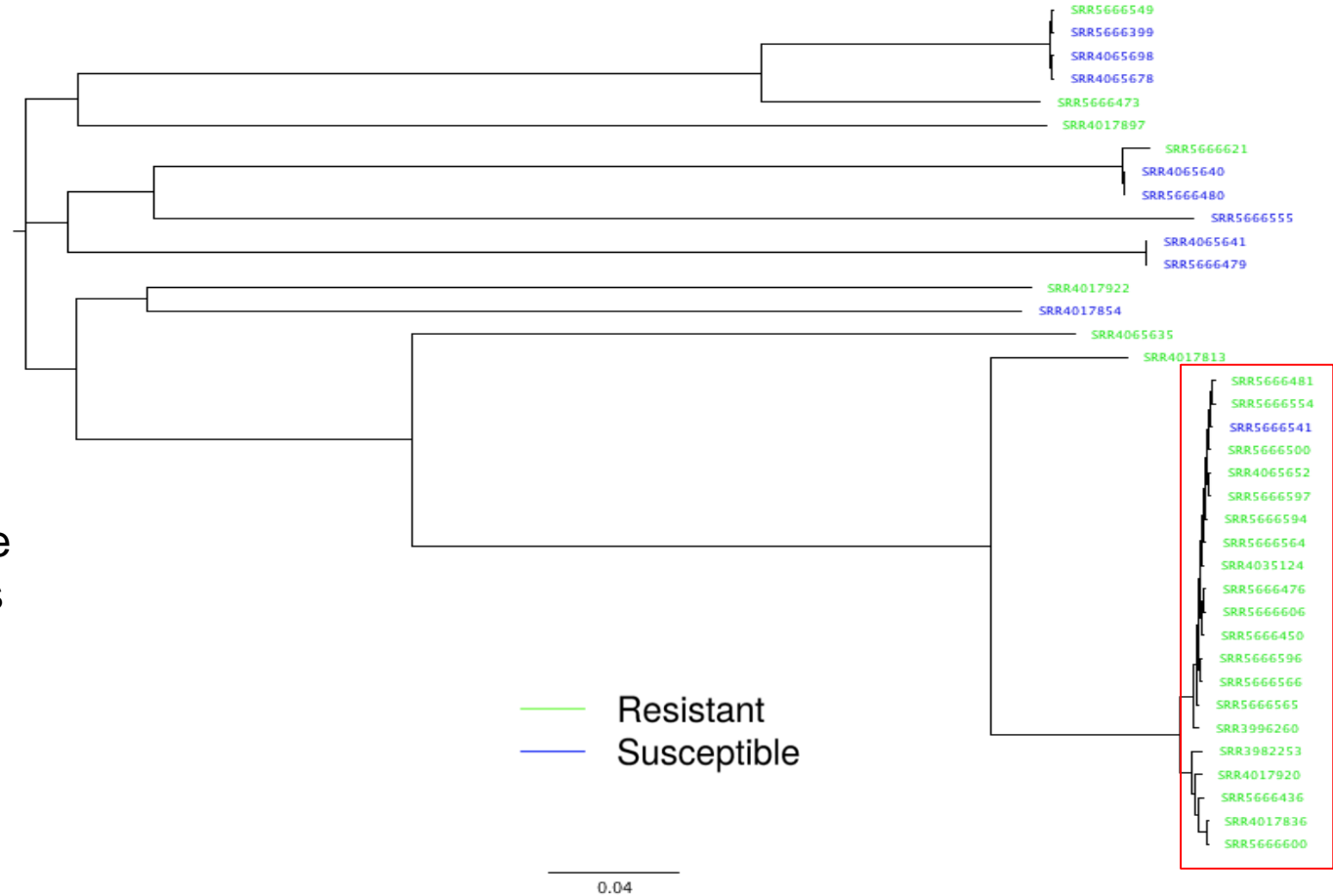
- without genome alignment
- without the need for a reference genome

kSNP3.0 - Results



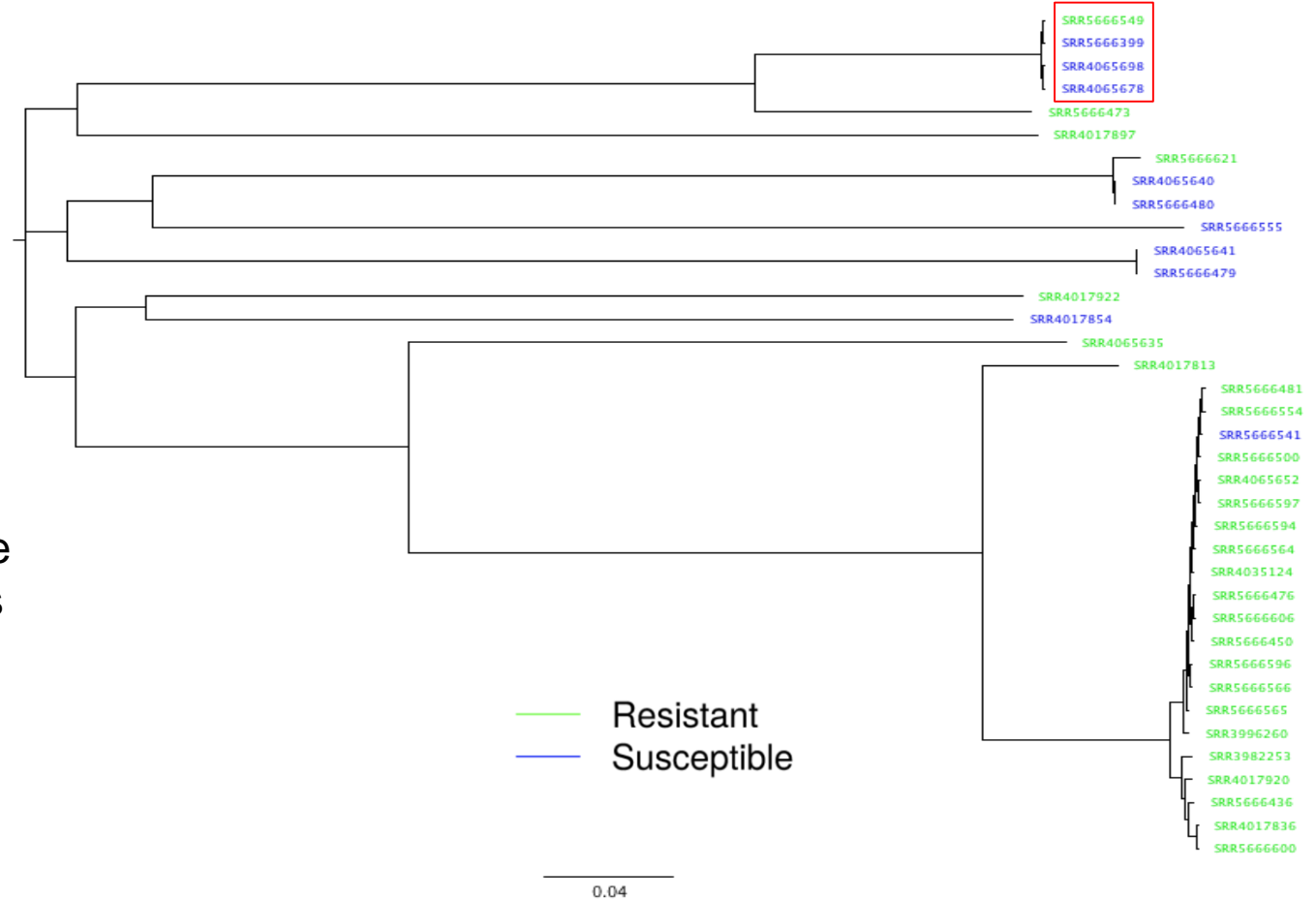
Analysis of Susceptible
and Resistant samples

kSNP3.0 - Results

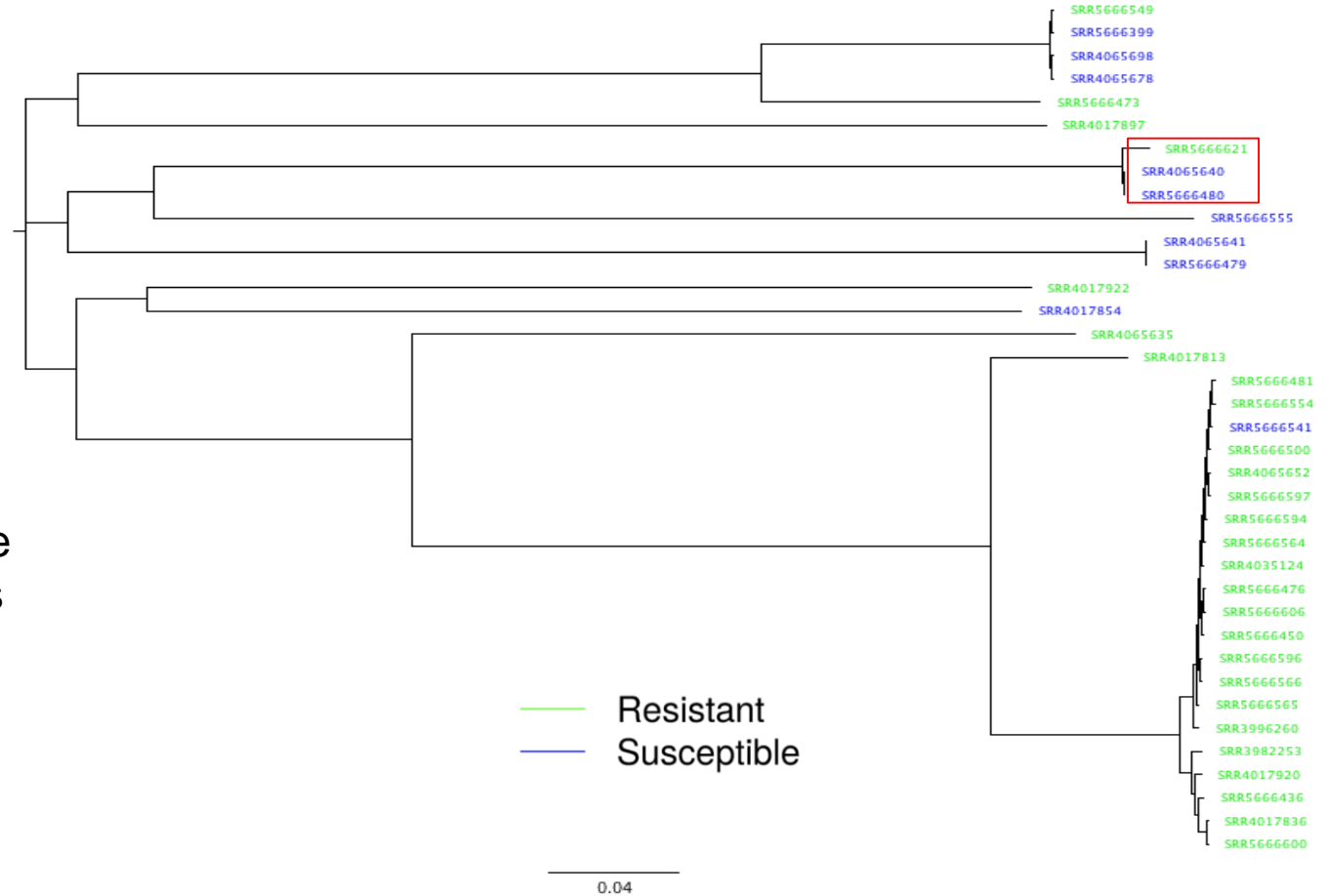


Analysis of Susceptible and Resistant samples

kSNP3.0 - Results



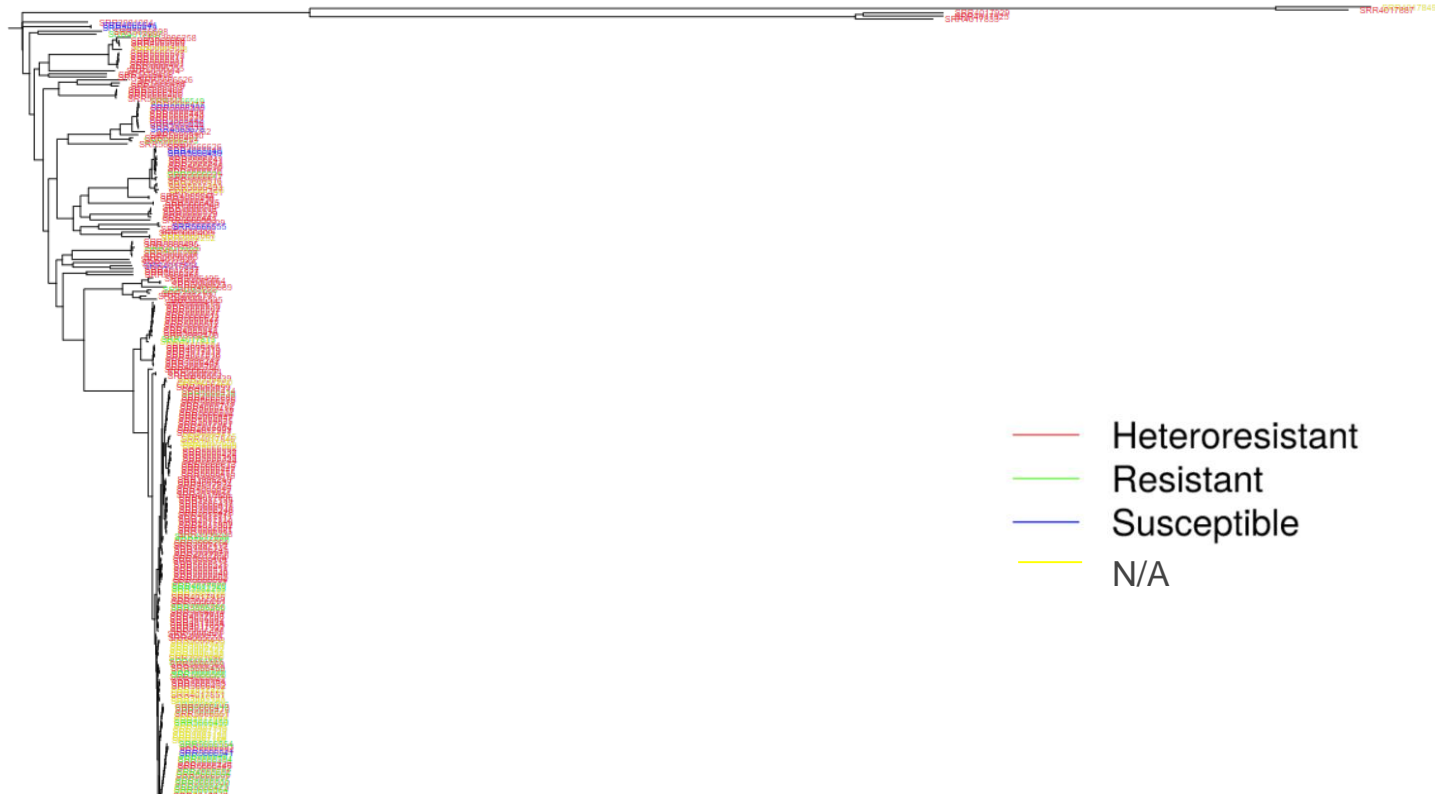
kSNP3.0 - Results



Analysis of Susceptible and Resistant samples

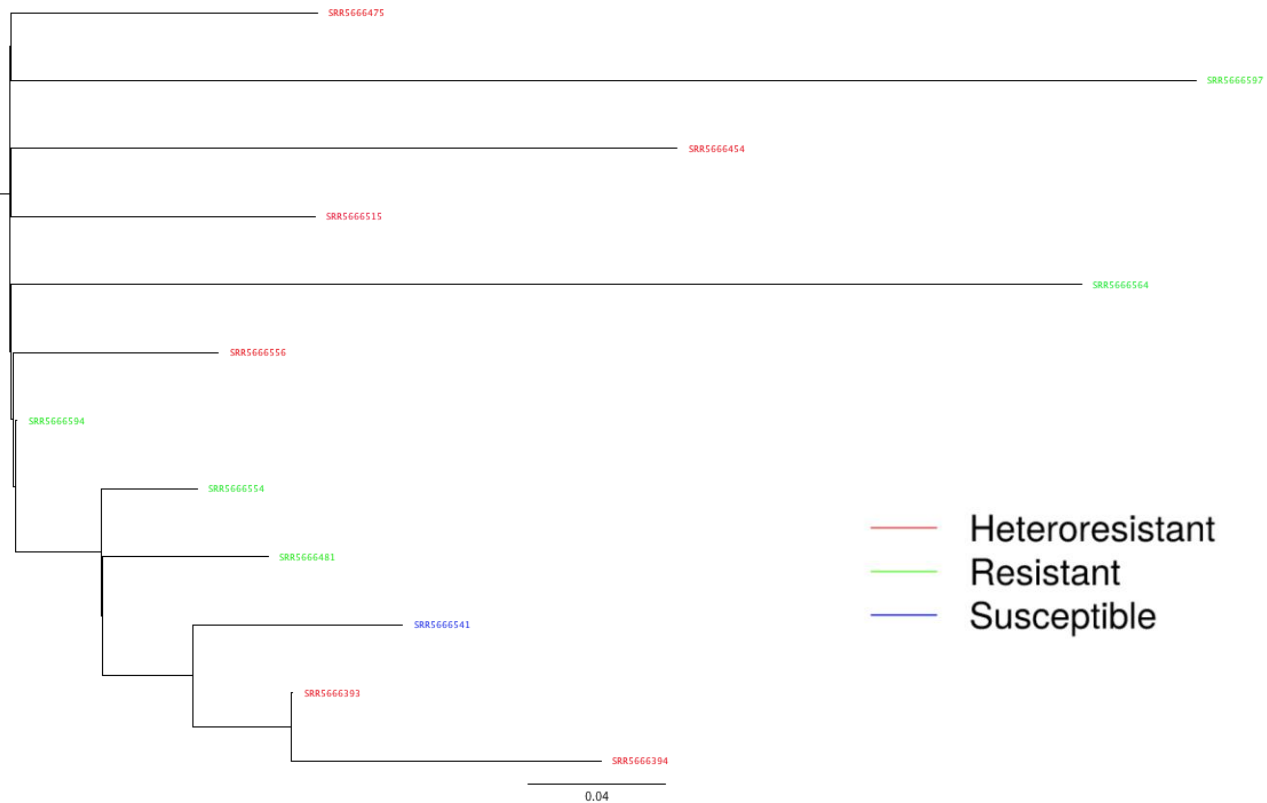
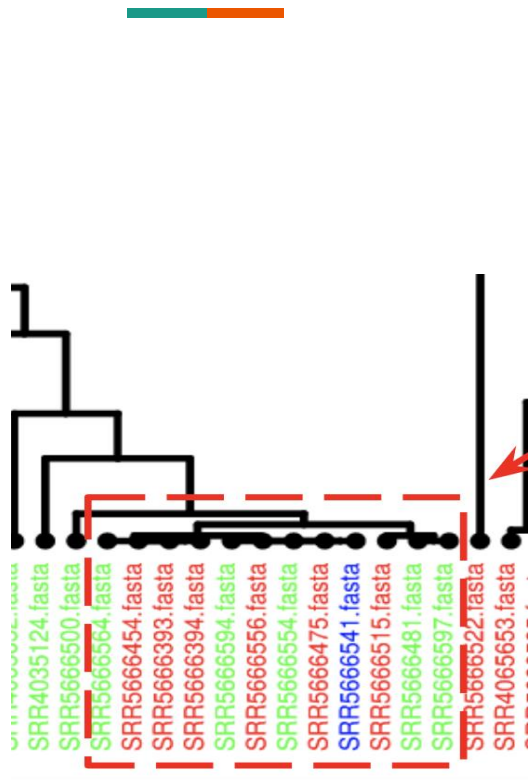
kSNP3.0 - Results

Whole Population



kSNP3.0 - Results

Cluster of Interest



Next Steps



Pan Genome:

- Results from Functional Annotation group to start of Pan and Core Genome Analysis
- Find presence and absence of genes in each group (R,H,S) ⇒ make list of unique genes
- Functional analysis of unique genes

Phylogeny:

- cgMLST? No usable profile available for *K. pneumoniae*
- SNP analysis based on the groups of interest more informative once we append annotations
- kSNP3.0:
 - Annotate cluster of interest
 - Investigate similar clusters
 - Implement pipeline which predicts significant SNPs