

DTU

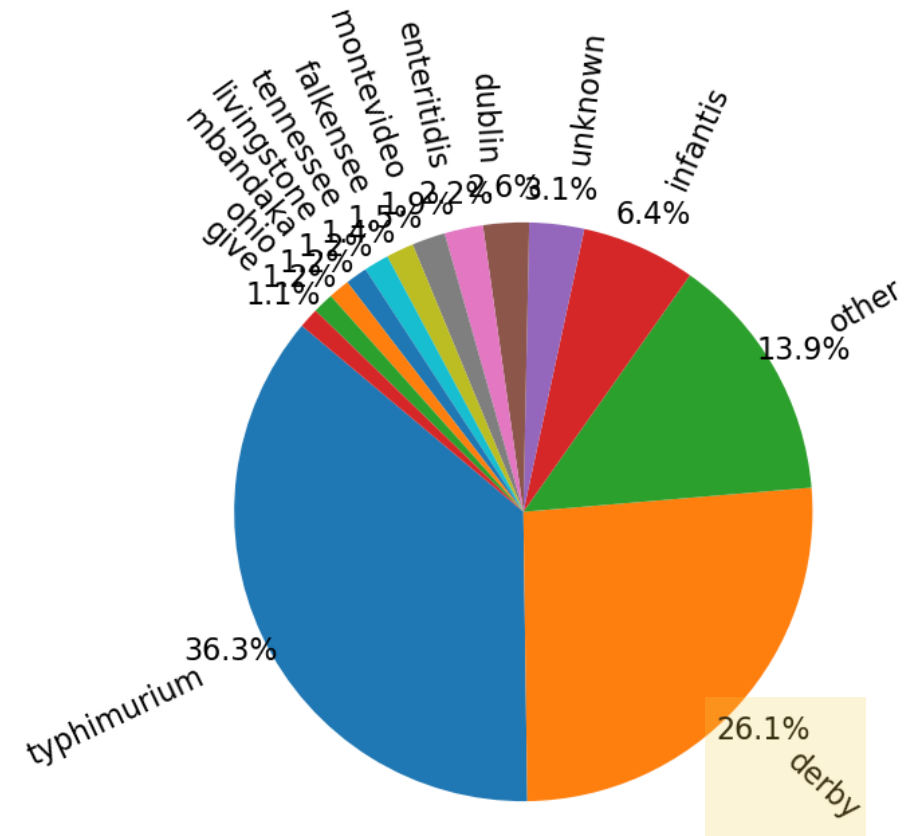


Salmonella Derby in Danish Pigs

Aim and Context

- S. Derby is one of the most prevalent serotypes in Danish pigs but is less frequently infecting humans.
- What does the landscape of S. Derby look like in Danish pigs?
How has it evolved?

Serotype Distribution of 4729 Salmonella samples from Animal/Food (FVST data)



Data

Whole Genome Sequencing Data of samples: 1634;
 1351 from Danish Food Authority (+279 from freezers)
 4 from Online source
 Illumina, Oxford Nanopore

- Increase in ST682? Shift from ST40 to ST682?

ST Types:

851 **ST682**

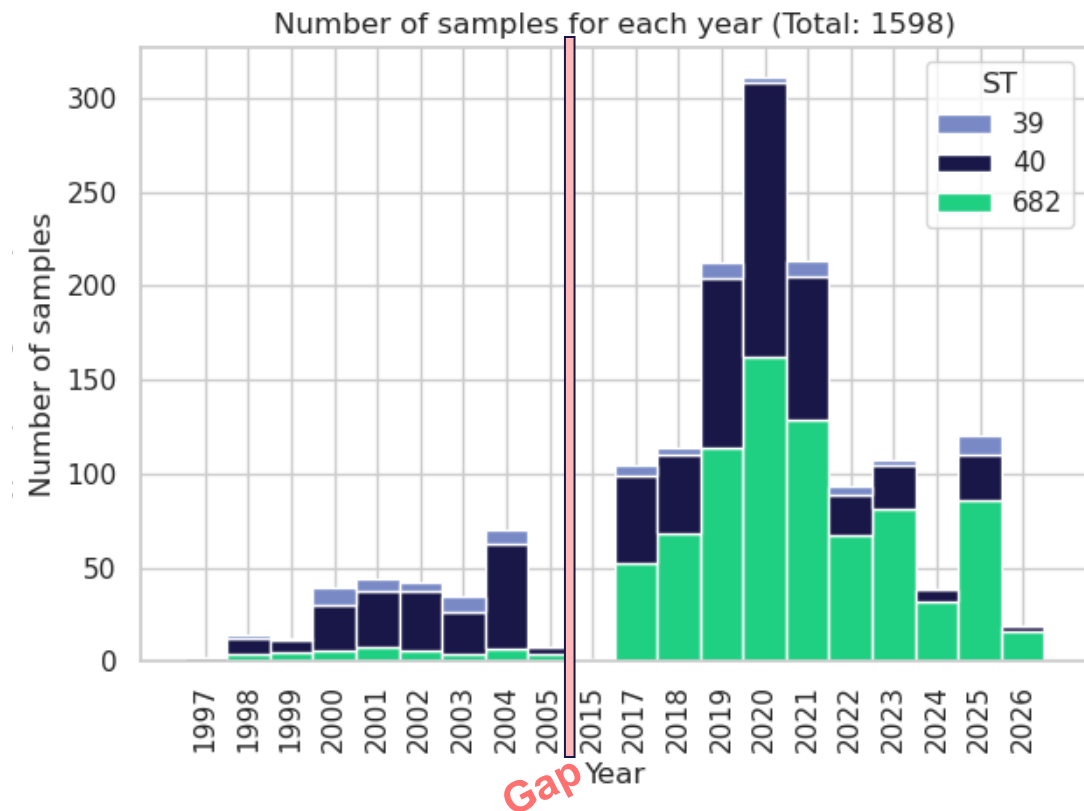
662 **ST40**

85 **ST39**

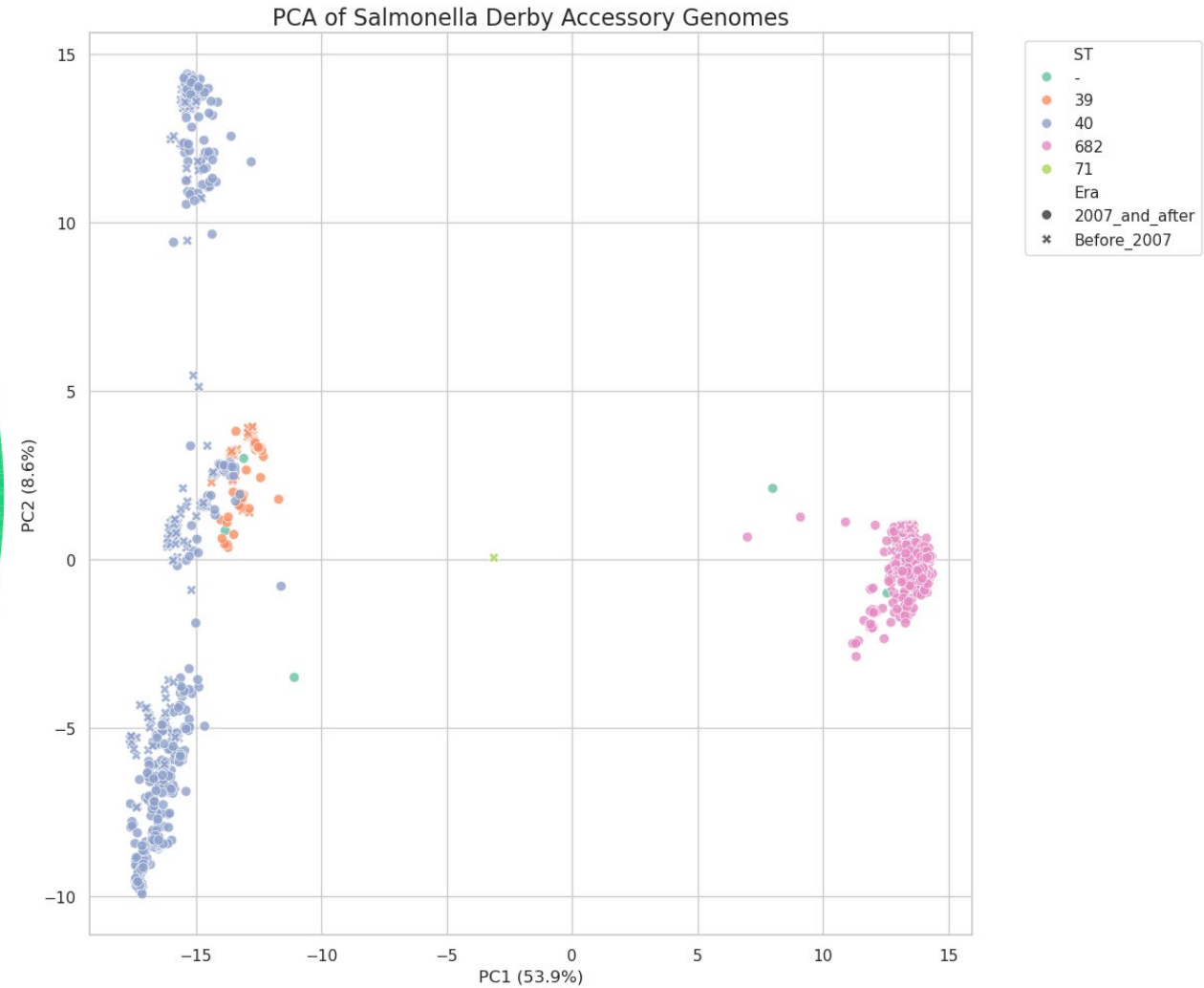
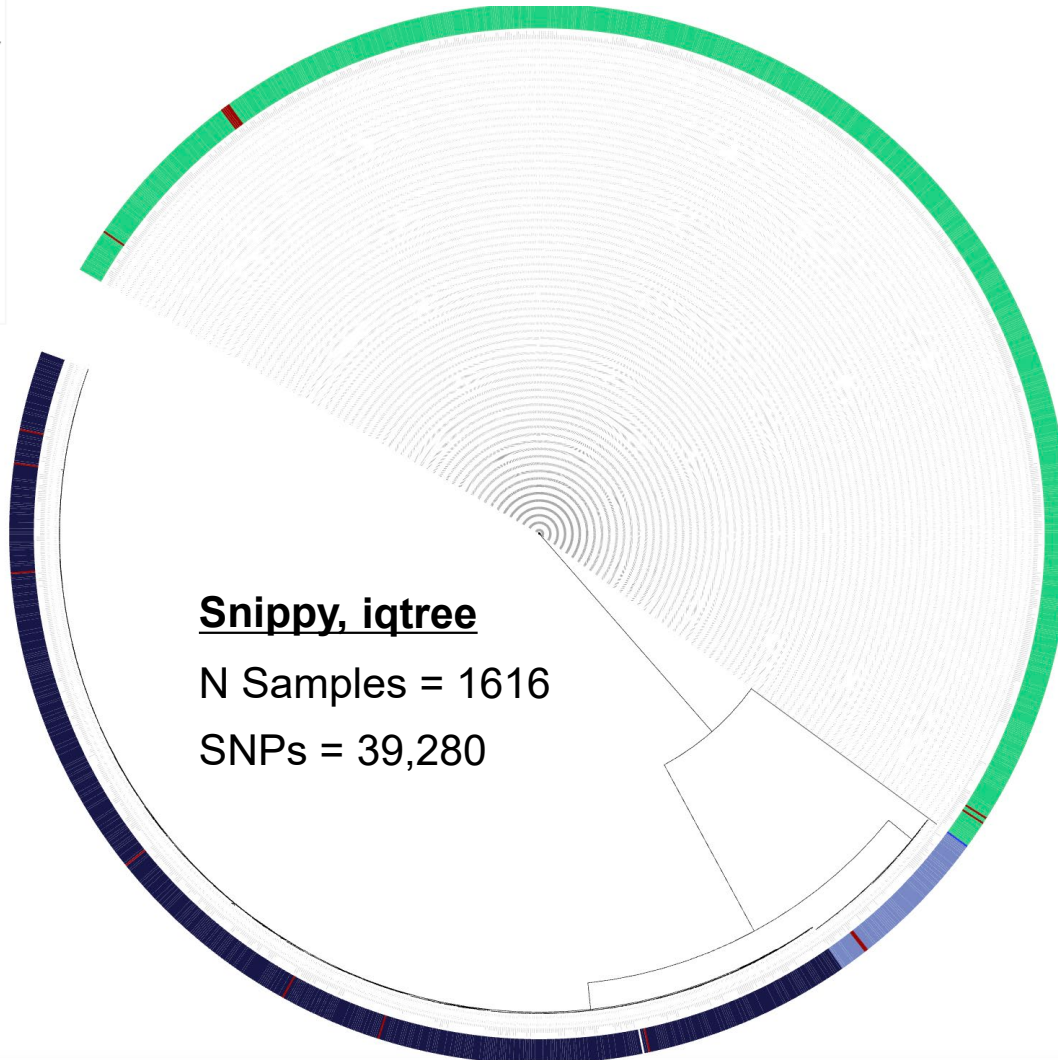
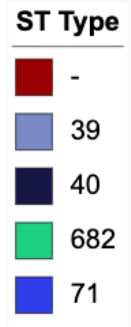
1 **ST71**

17 **ST-**

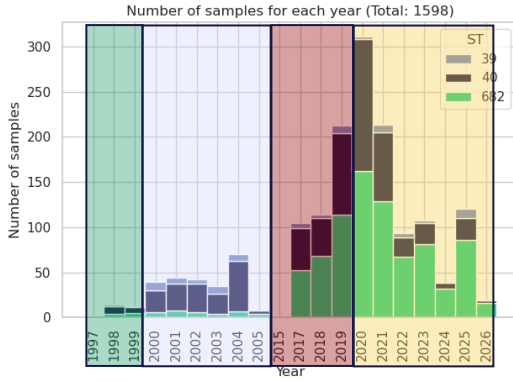
= **1598 samples**



Clustering on Sequence Type



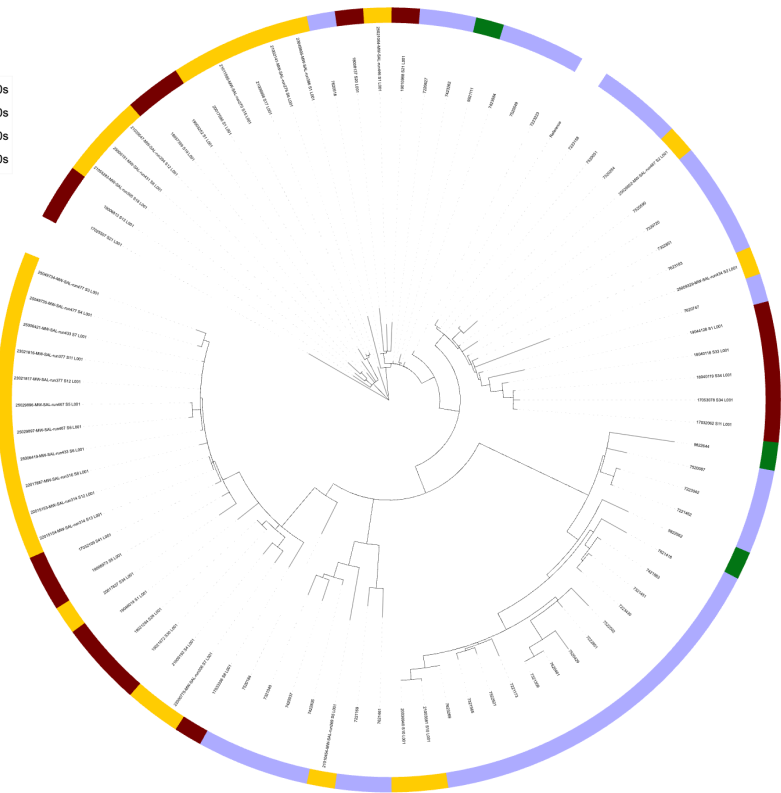
Snippy Trees: Clustering on years; ST39+ST40



ST39

N Samples = 85
SNPs = 899

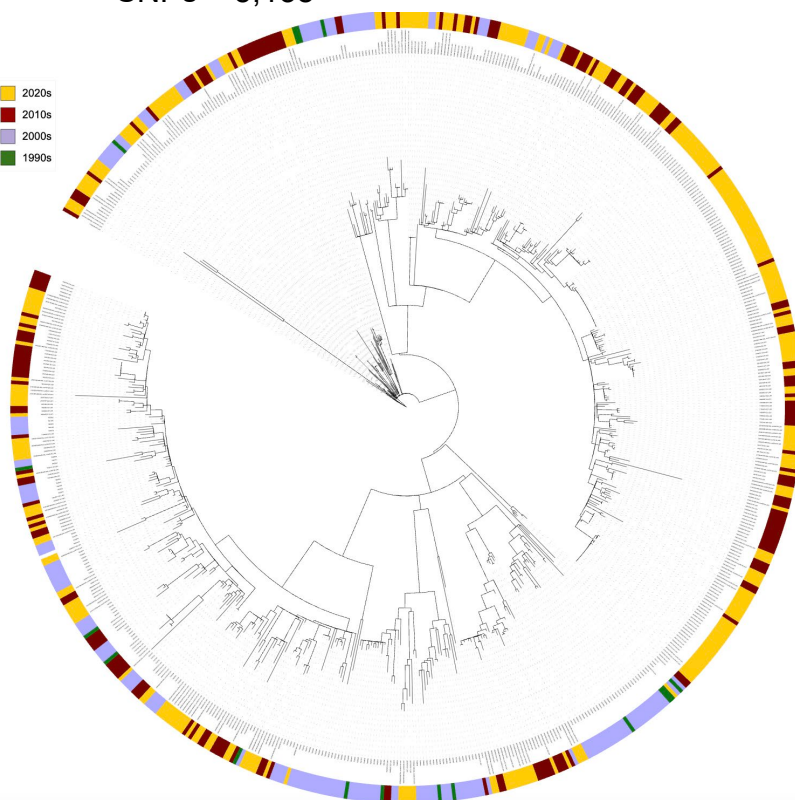
2020s
2010s
2000s
1990s



ST40

N Samples = 662
SNPs = 5,138

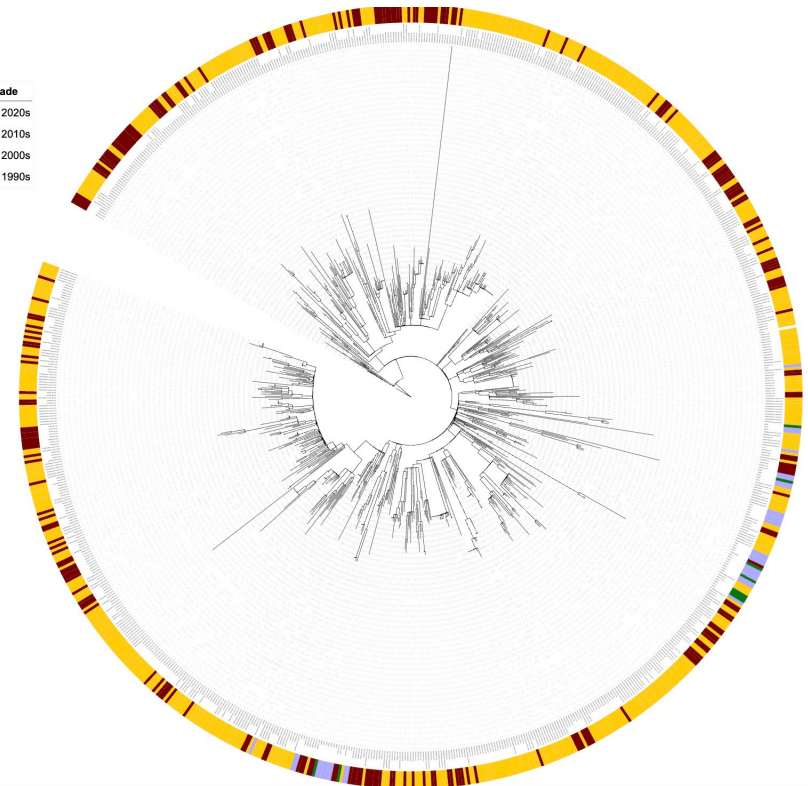
2020s
2010s
2000s
1990s



ST682

N Samples = 851
SNPs = 5,075

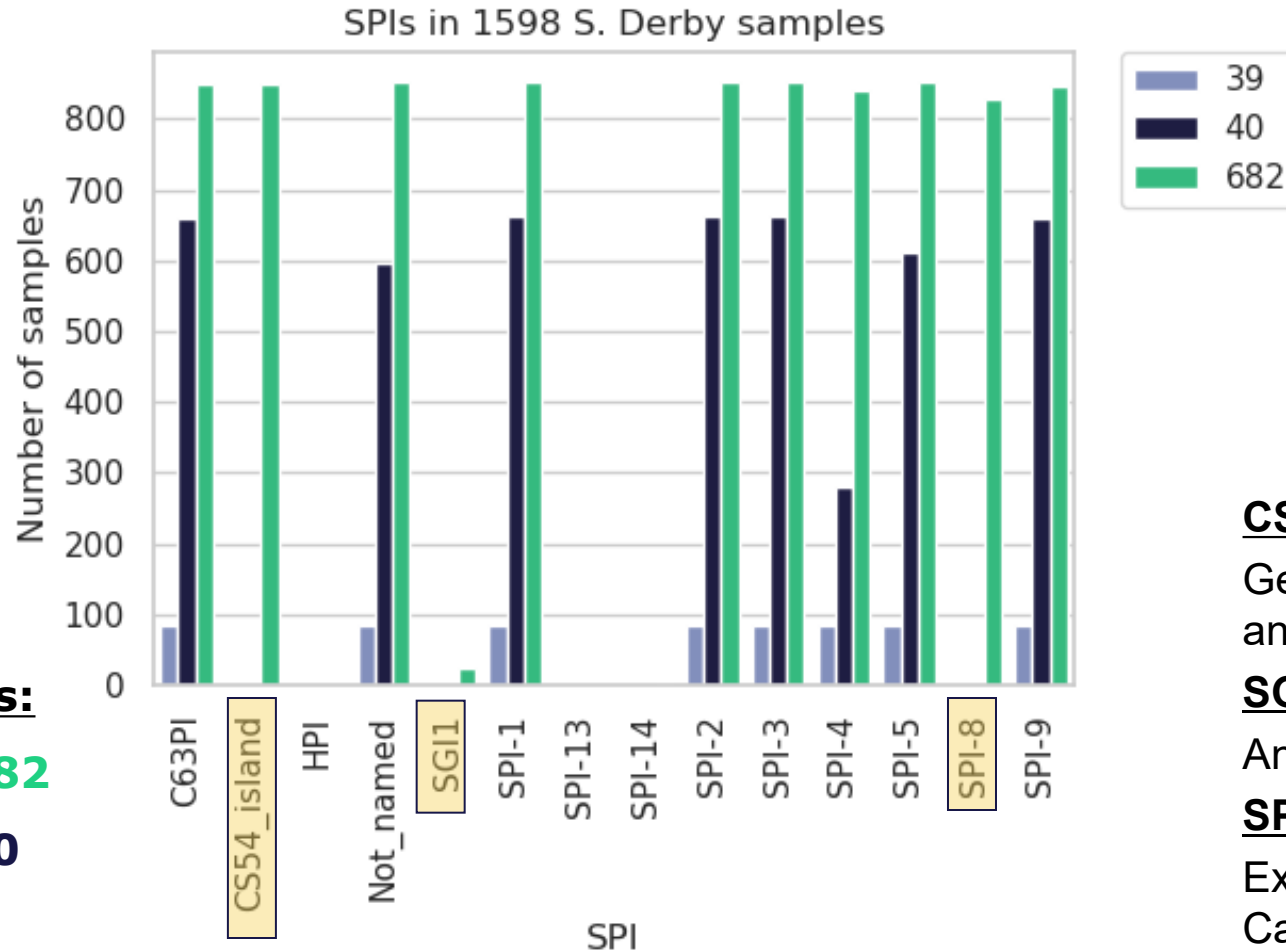
Decade
2020s
2010s
2000s
1990s



What do the genomes contain?

Salmonella Pathogenic Islands:

More SPIs in ST682 compared to ST40 and ST39

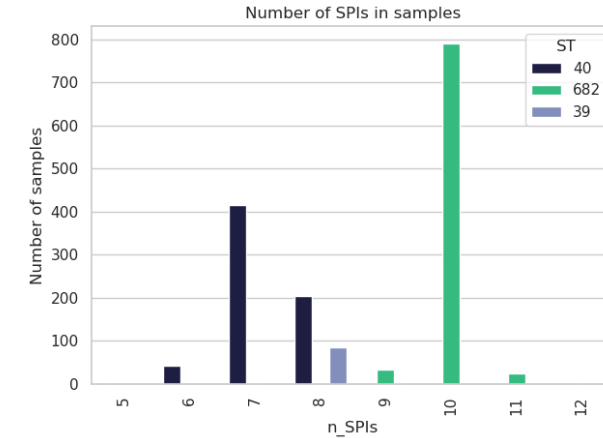


ST Types:

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CS54 Island:

Genes important for intestinal colonization and fecal shedding

SGI-1:

Antimicrobial Resistance Genes

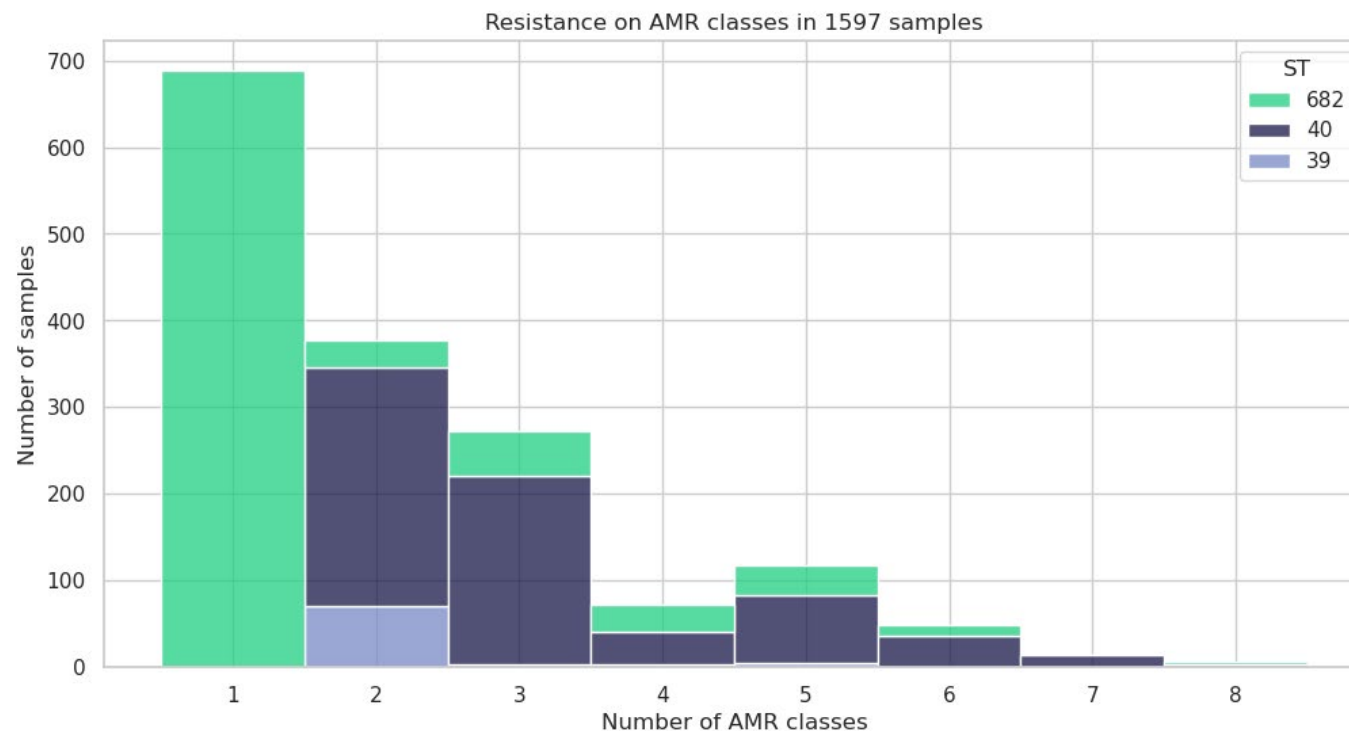
SPI-8:

Exact biological function is unknown.
Carries two bacteriocin pseudogenes

Antimicrobial Resistance Classes:

More MDR in ST40 samples compared to ST682

ARGs mapped to AMR classes using ResFinder database.



ST Types:

851 **ST682**

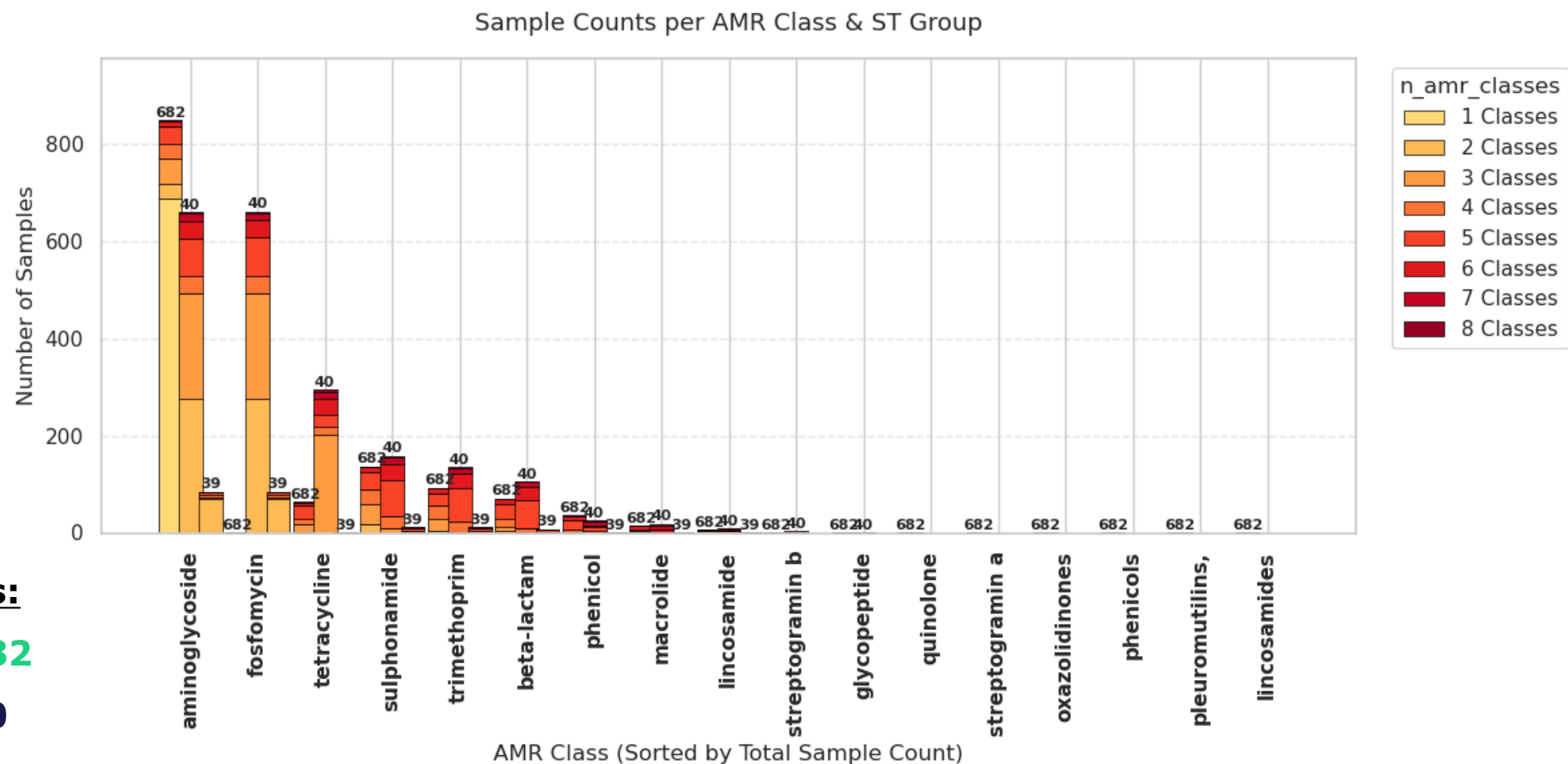
662 **ST40**

85 **ST39**

One ST682 sample from 2018 has no resistance genes.

Antimicrobial Resistance:

Fosfomycin resistance in ST40, few ST682 with unique class



ST Types:

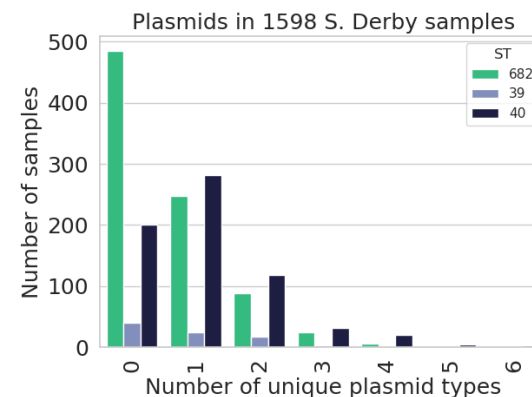
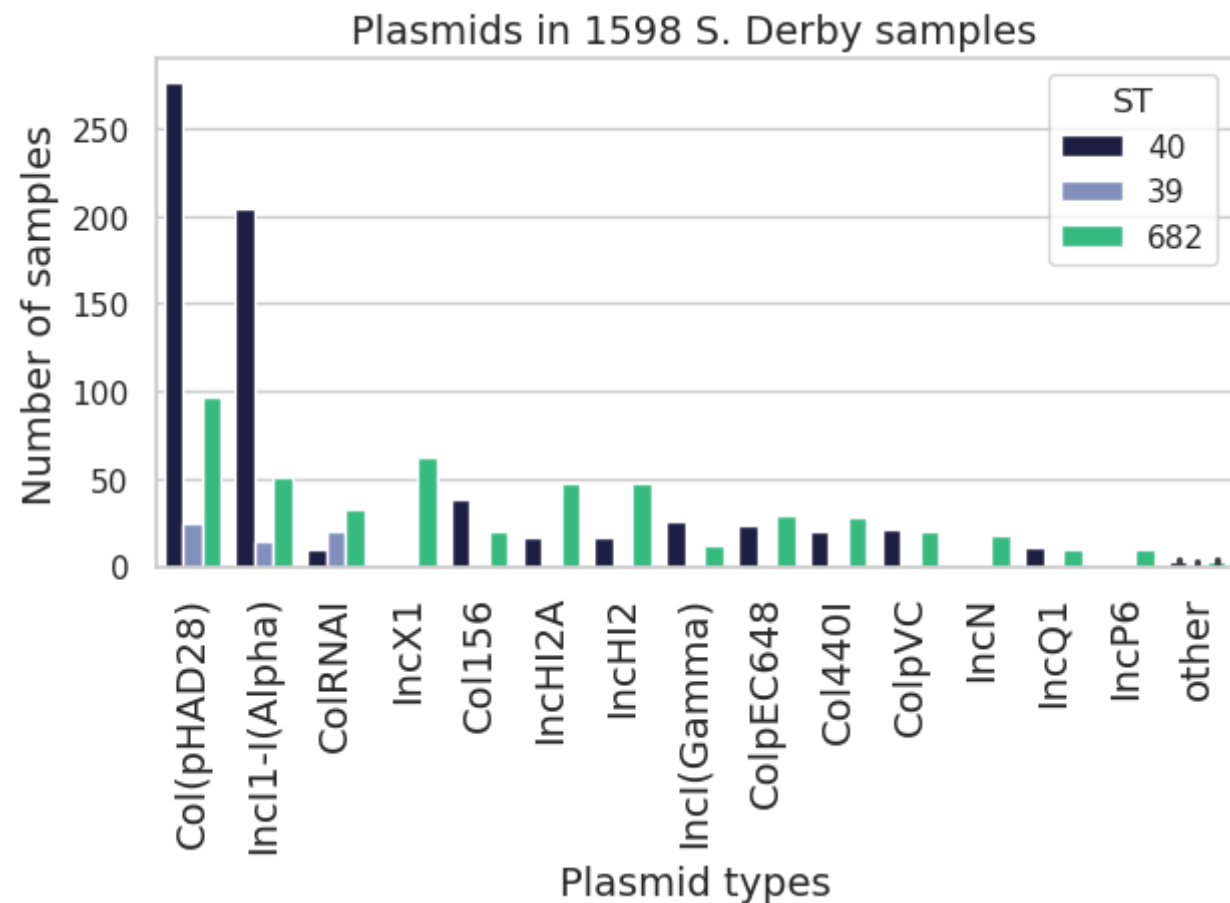
851 **ST682**

662 **ST40**

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Plasmids:

Many ST682 samples without plasmids



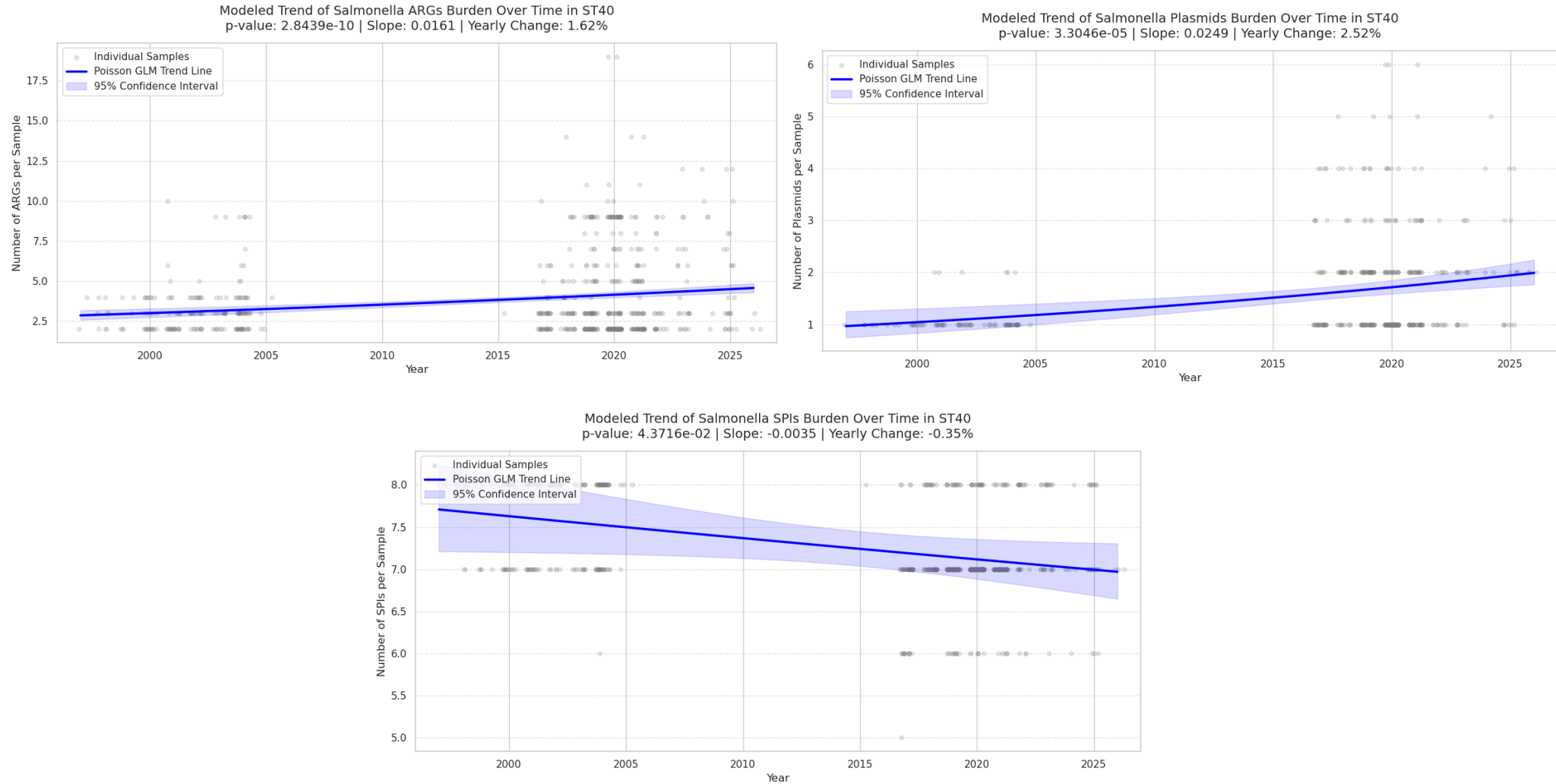
ST Types:

851 **ST682**

662 **ST40**

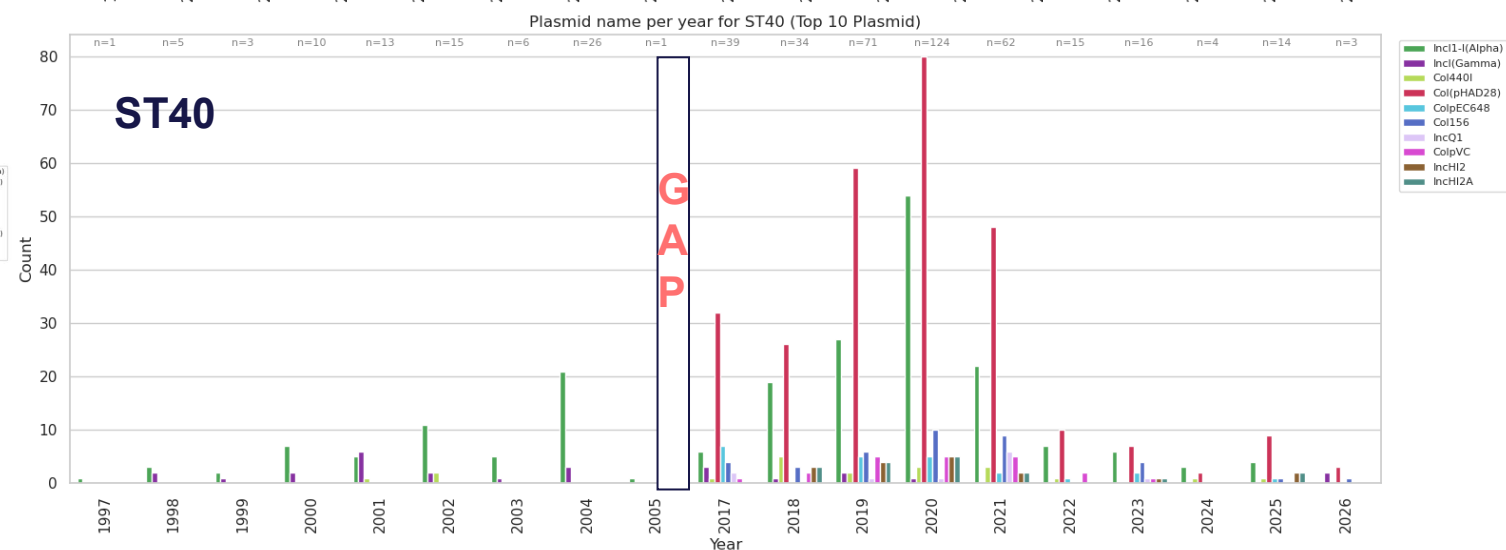
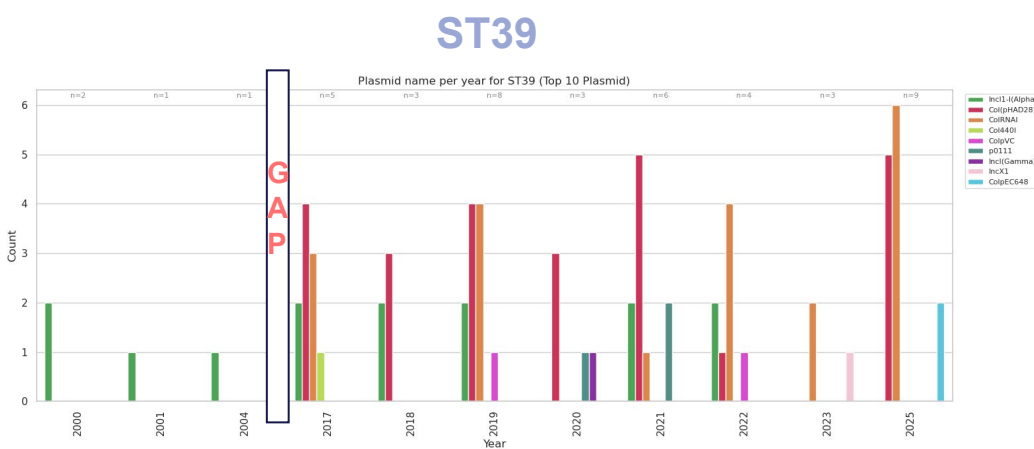
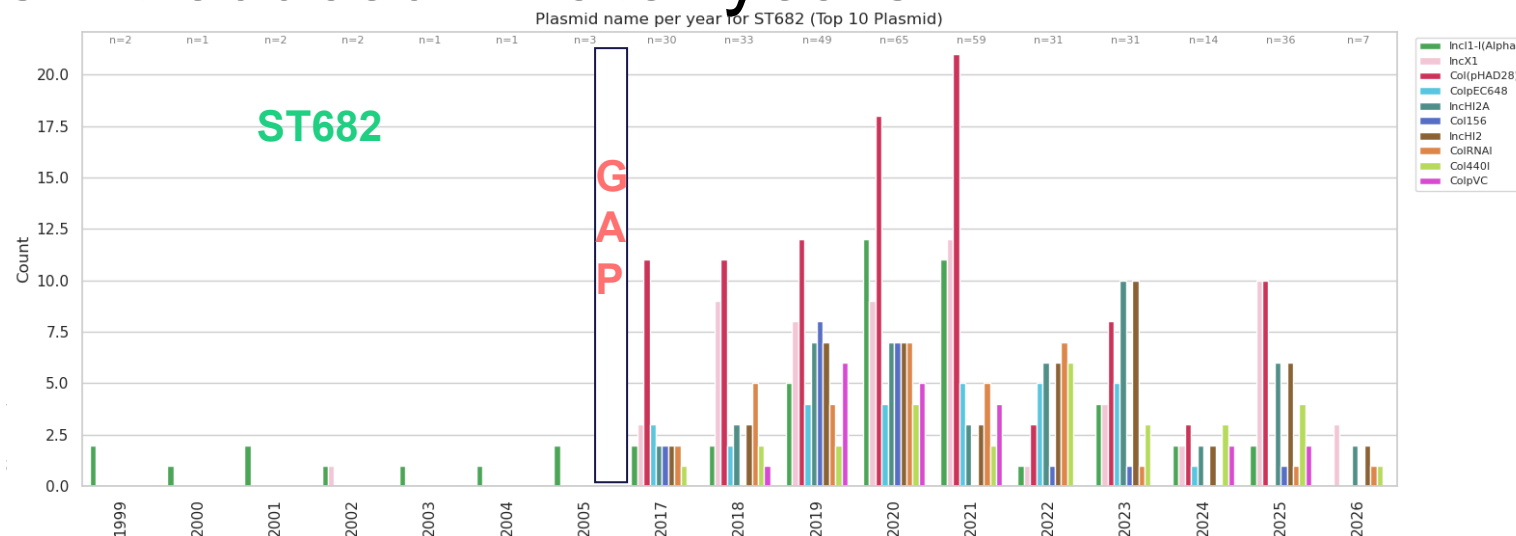
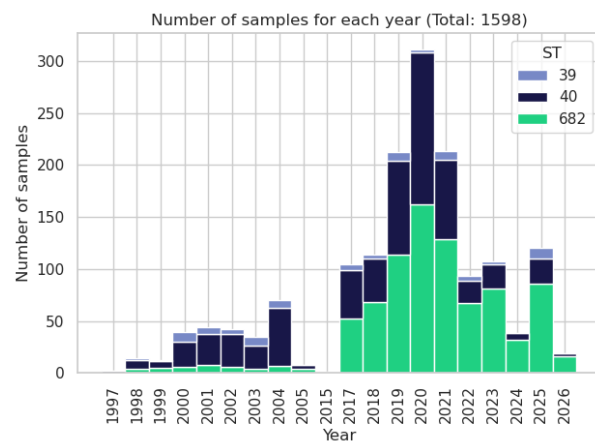
85 **ST39**

ST40 phenotypes evolve significantly with time

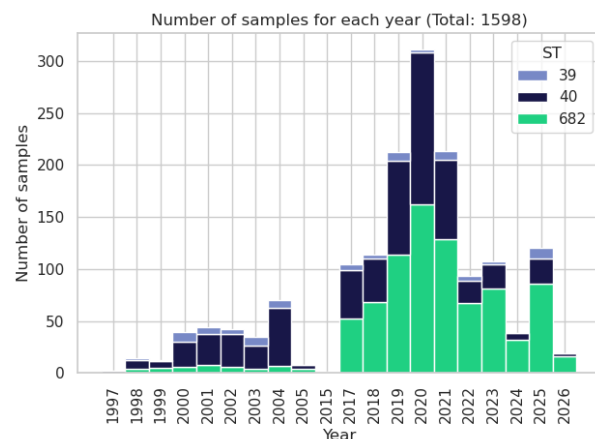


Plasmids:

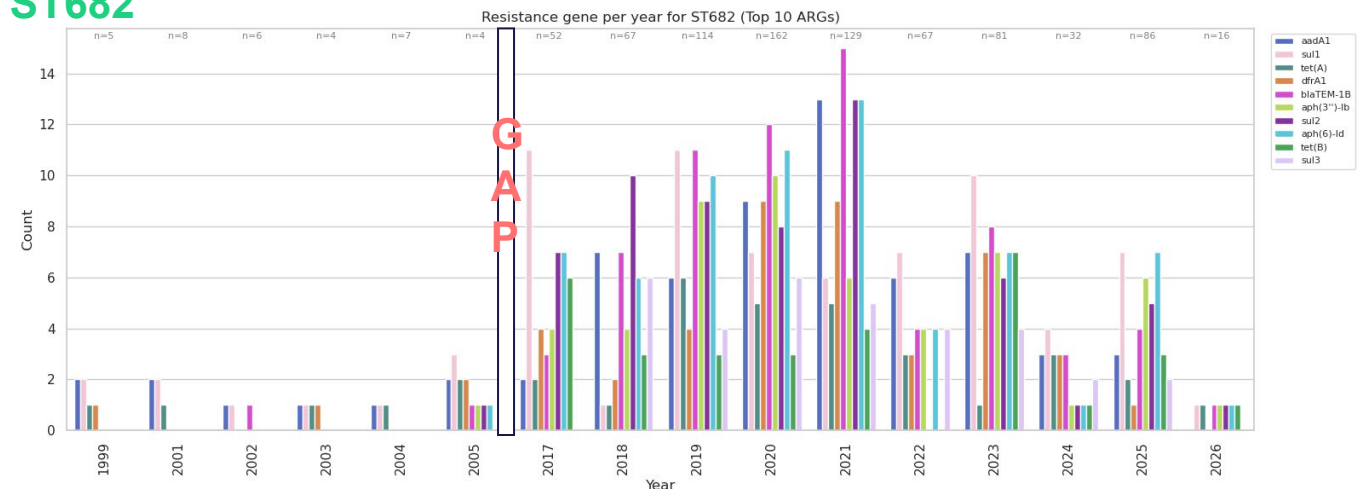
More plasmid types introduced in later years



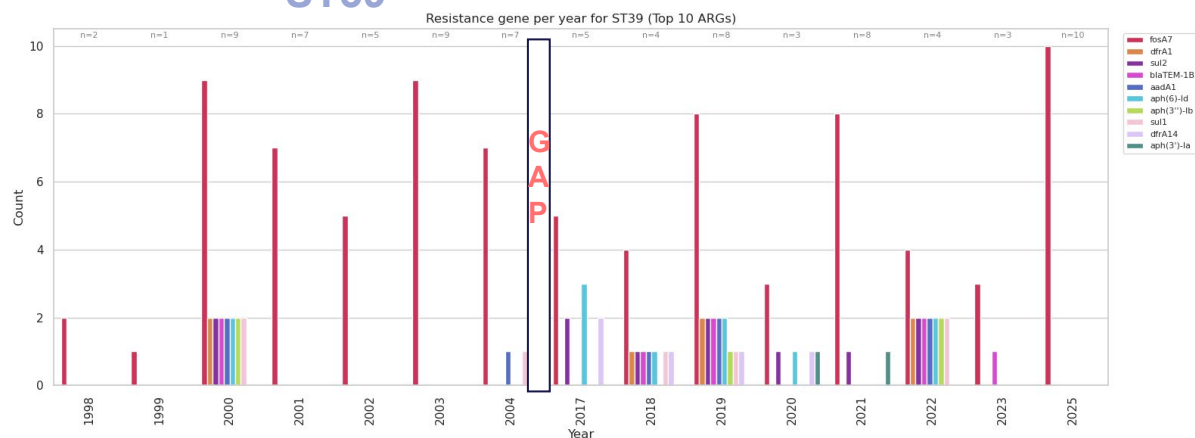
Antimicrobial Resistance: More ARGs introduced with time



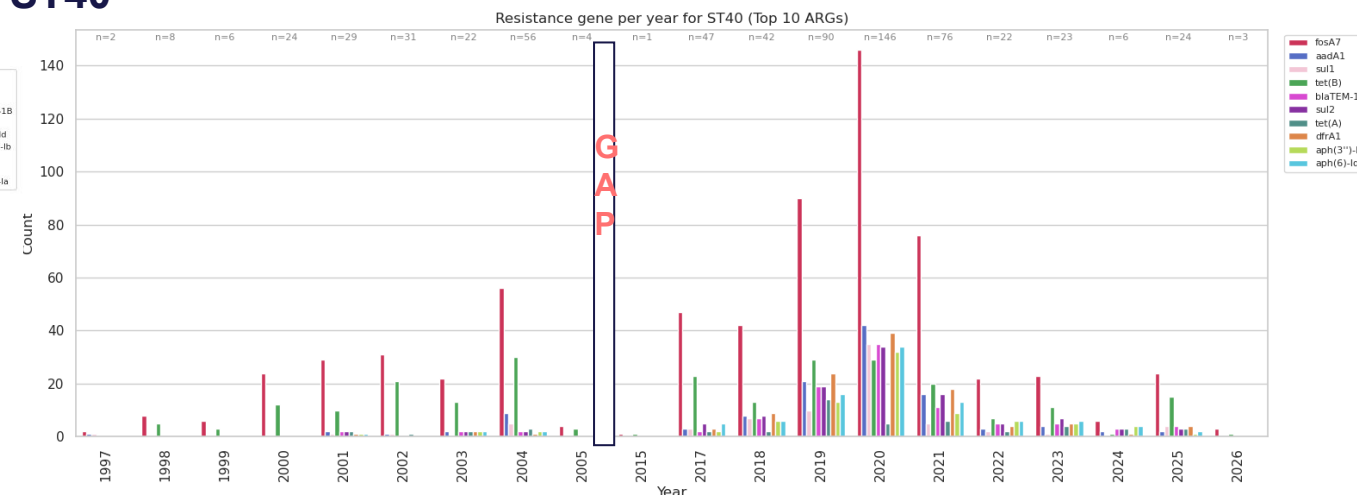
ST682



ST39



ST40



GWAS

Prokka, PseudoFinder, Roary, Scoary.

ST39 + ST40

- **Nutritional Versatility:**
Feed of carbon sources such as myo-inositol, galactitol, D-Xylose, Sugar Acids.
- **Fosfomycin resistance**

Stress Adaptation:

- Oxidative Stress sensing/regulation (perR)
- Plasmid maintenance and toxicity; CcdB
- CRISPR (casE) defense against phages.

----- F I L T E R S -----

Sensitivity threshold: 90%

Specificity threshold: 90%

p-value threshold: 0.05 (benjamini)

----- R E S U L T S -----

Number of genes enriched in ST682: 89

Number of genes enriched in ST40eBG57: 67

ST682

- **Intracellular replication** (Salmonella-containing Vacuole (SCV)):
SPI-2 Type III secretion effectors (sifA, sspH1, sspH2)
- **Mucosal Attachment**

Host immunity evasion:

- Flagellar **phase variation** (hin)
- **Quorum Sensing** (Isr operons); Biofilm formation.

Stress Adaptation:

Toxin-Antitoxin (TA) Modules.

Persister cells; Antimicrobial Tolerance?

BEAST Bayesian Skyline

1930: Expansion of **ST40**

1980-2000: SLOW expansion of **ST39**

2005: Zinc oxide was made prescription only.

2007: Expansion of **ST682**

2009: Update to Salmonella Surveillance method; From MixELISA to microbiological tests.

2010: Expansion of **ST40**

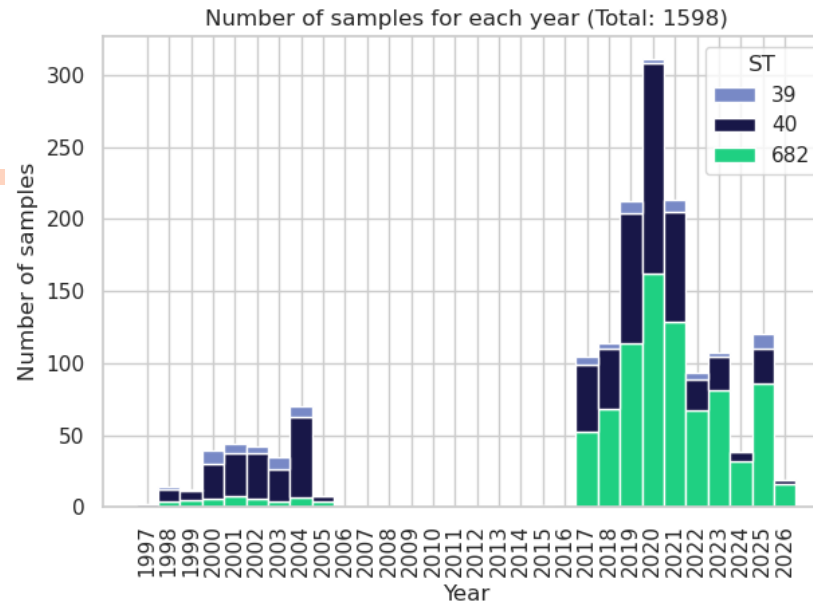
2010: Yellow card control of AMR use implemented.

2012: Expansion of **ST682**

2018: Reduction of **ST39**

2019: Reduction of **ST40**

2020: Reduction of **ST682**

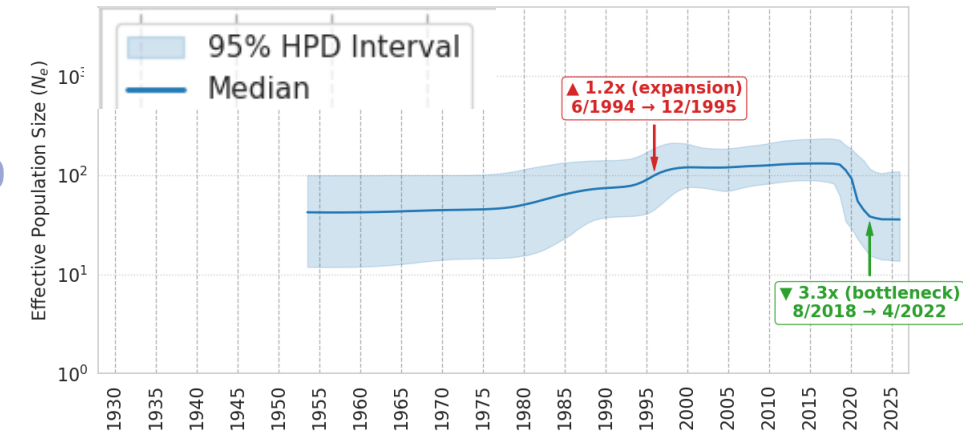


Site model: HKY

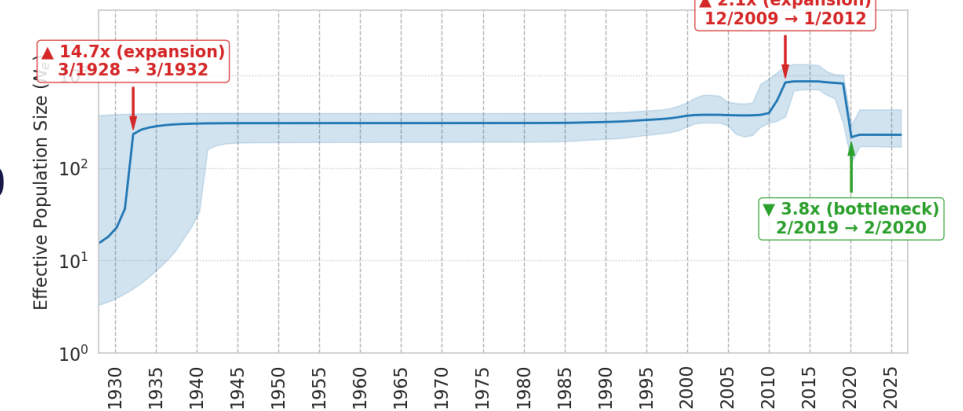
Strict Clock

ClockRate LogNormal

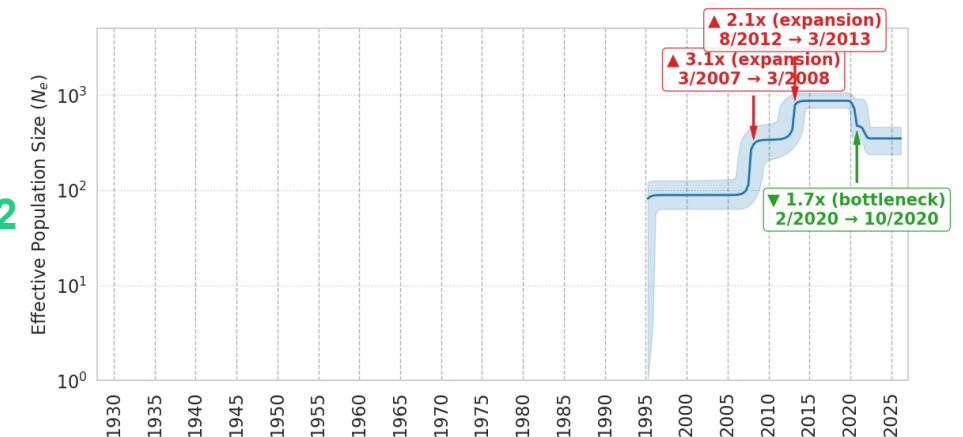
ST39



ST40



ST682



Conclusion

- Indication of shift from ST40 to ST682.
- ST682 form different cluster from ST39 / ST40
- ST39/ST40
 - has more Plasmids/AMR than ST682 (fosA).
 - More diverse metabolism. CRISPR CasE defense.
- ST682;
 - More SPIs than ST40
 - better at mucosal colonization. Better fecal shedding. (CS54 Island)
 - Immune evasion; Biofilm, antigen flipping, TA Modules; Persister cells (instead of ARGs?).
- BEAST:
 - Effective sample size reduction around 2020 corresponds to sample size.
 - No obvious explanation for expansions / reductions.