

Phylogenomic analysis of *Campylobacter jejuni* isolated from gastroenteritis cases in Michigan

Jose A. Rodrigues¹, Heather Blankenship^{1,2}, Wonhee Cha¹, Rebekah Mosci¹, Shannon D. Manning¹

¹Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan
²Michigan Department of Health and Human Services, Bureau of Laboratories, Lansing Michigan

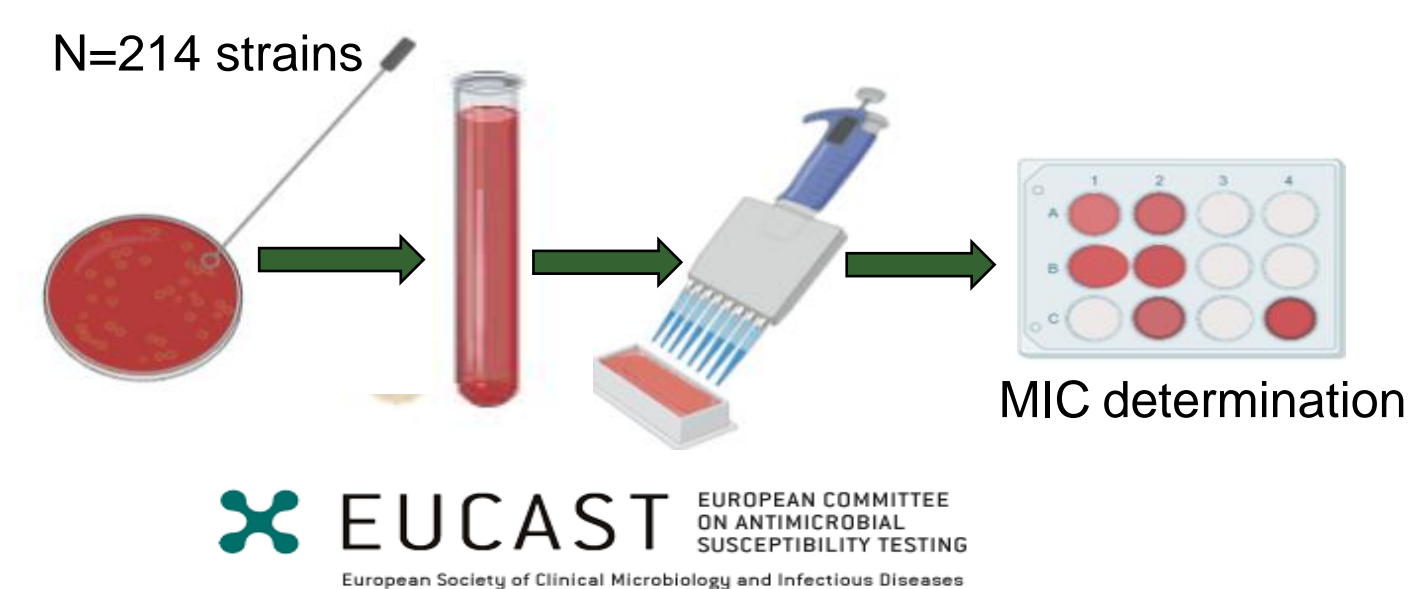
Contact Information:
Jose Rodrigues
Michigan State University
Email: rodr651@msu.edu
@JoseARodrigues8

INTRODUCTION

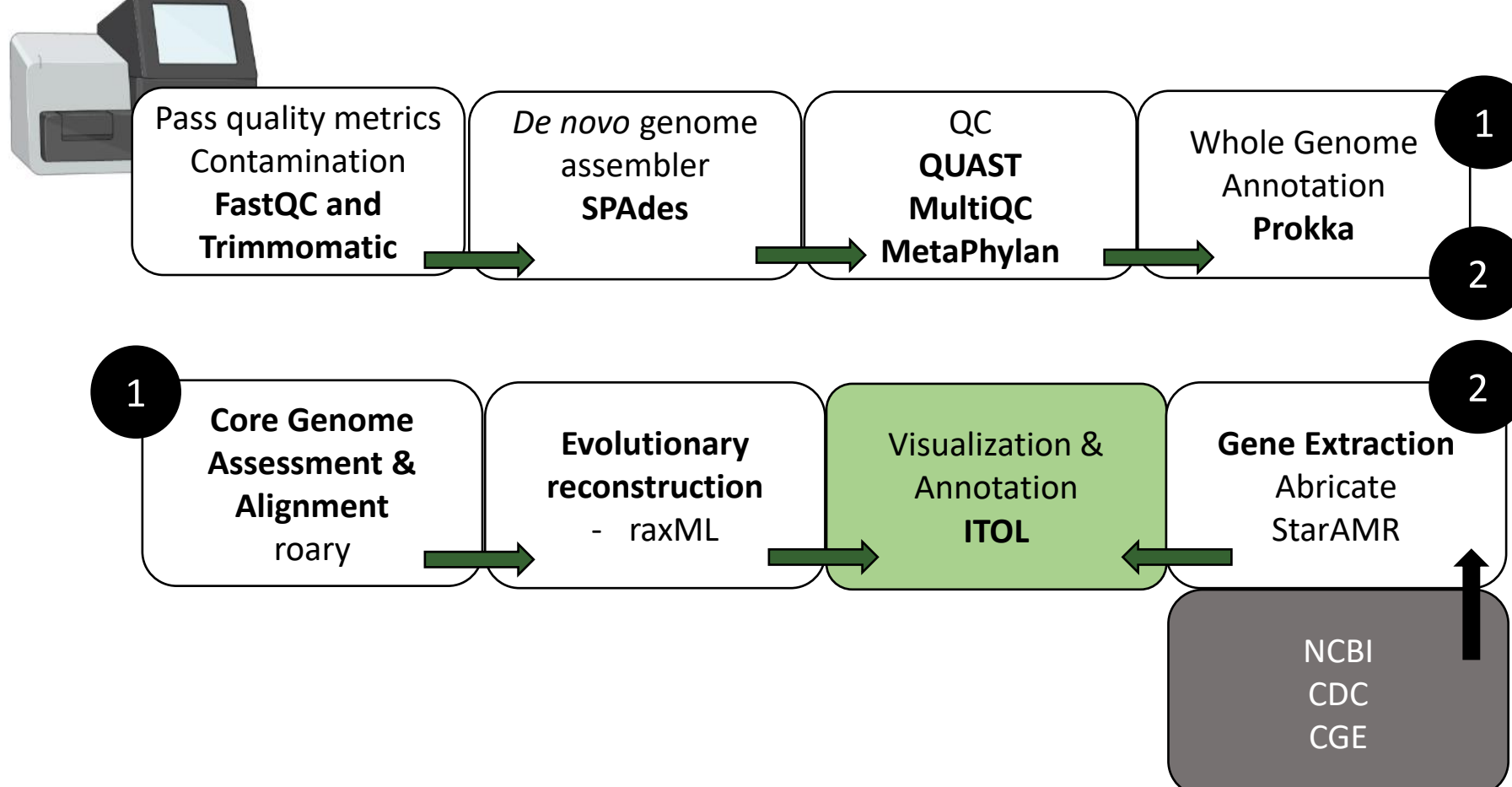
- C. jejuni* is the leading cause of bacterial gastroenteritis worldwide, and the leading cause of bacterial foodborne illness in the United States¹
- Bacterial typing methods can be used to confirm epidemiologic linkage and outbreak investigation²
- Use of whole-genome sequence (WGS) can enhance our ability to detect disease, understand transmission, ecology, evolution of enteric pathogens and assist in outbreak investigations³

METHODS

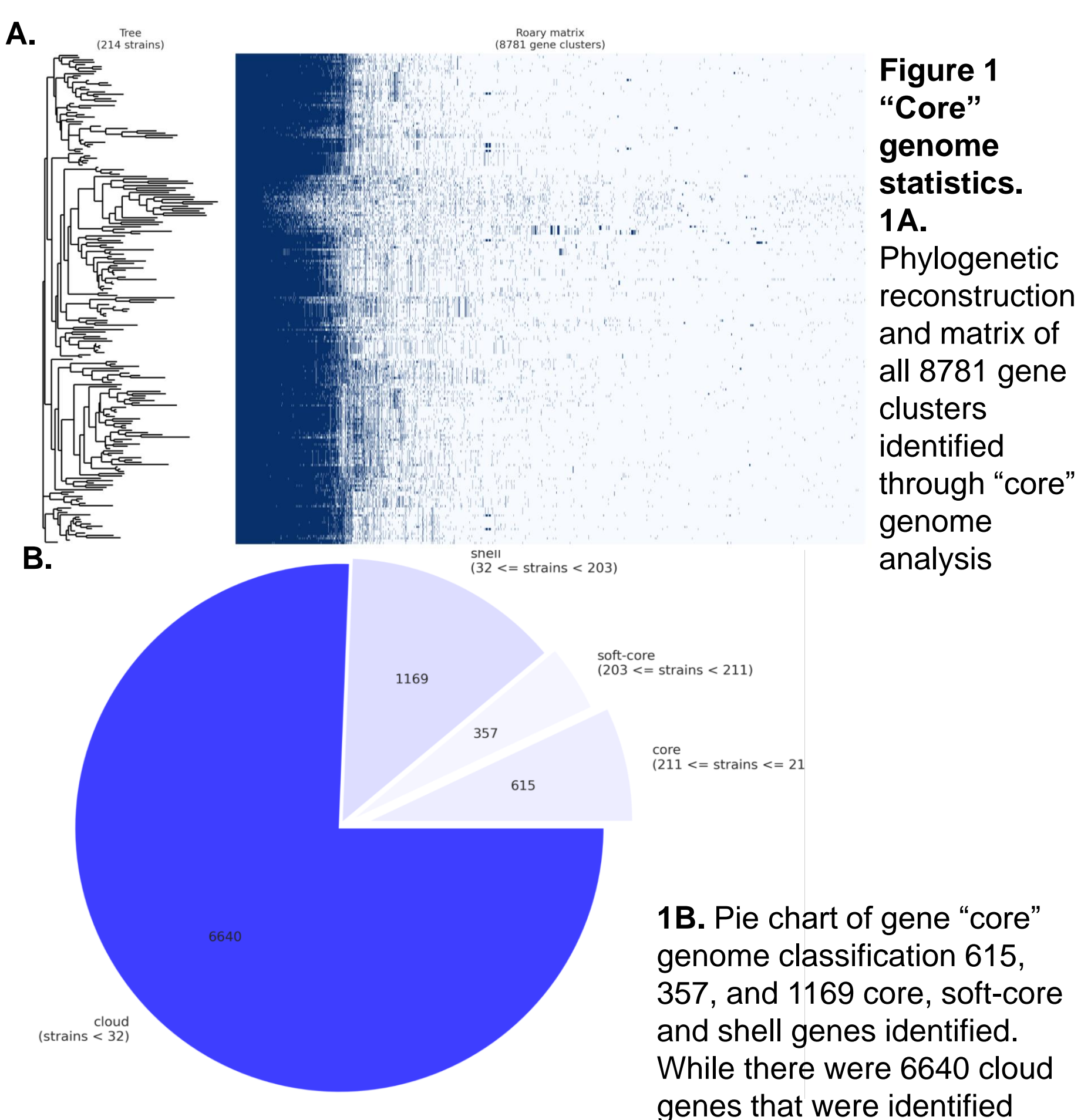
Campylobacter Isolates from 2011-2014 Sentinel surveillance in Michigan



Bioinformatics Pipeline:



RESULTS



"Core" genome multilocus sequence typing detects five unique clades of *Campylobacter jejuni*

RESULTS

Tree scale: 0.01

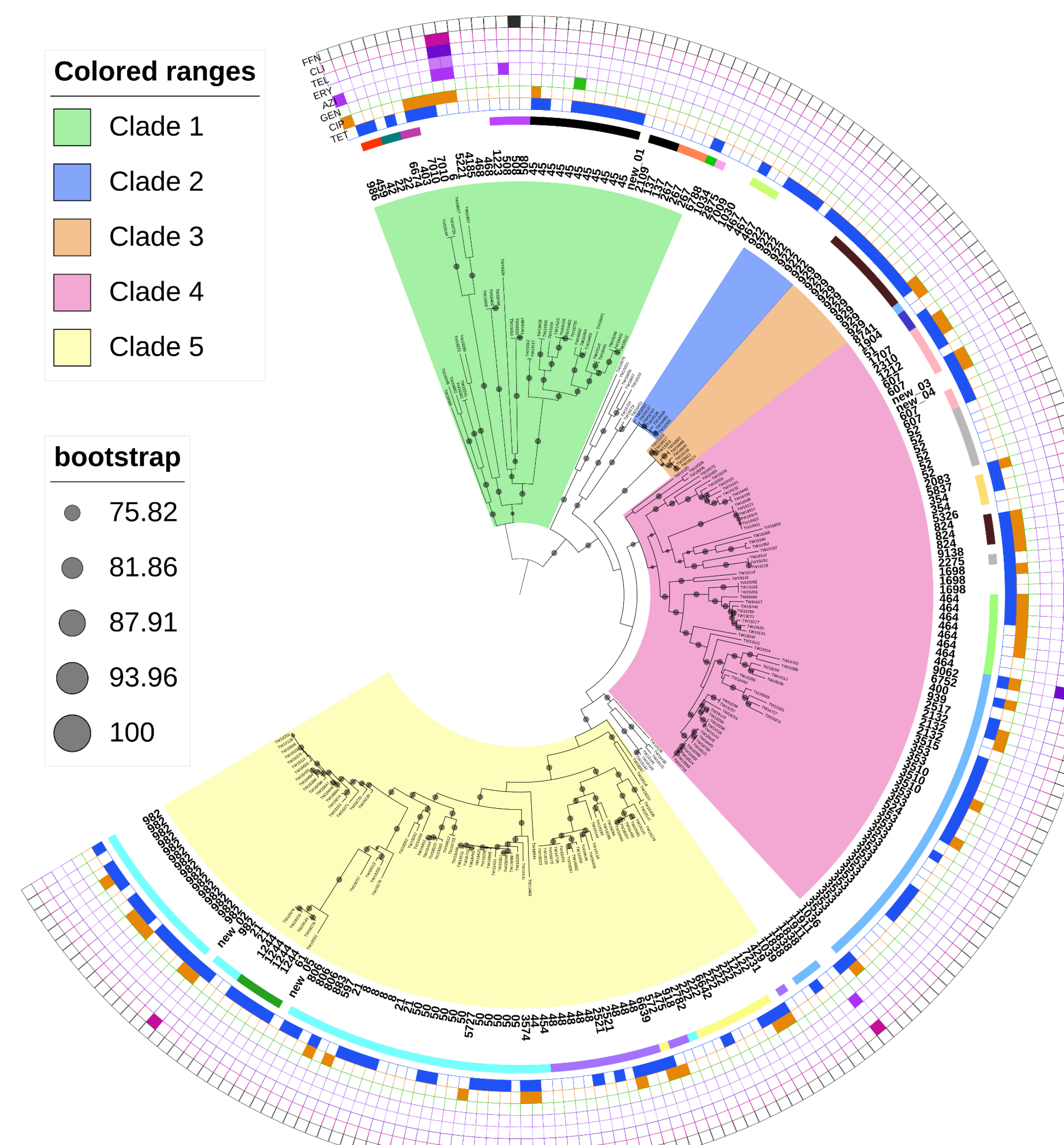


Figure 2. Maximum Likelihood Phylogeny of 615 "core" genes annotated with resistance phenotypes, sequences types (STs), and clonal complex (CC). Isolate IDs are found at each branch. Bootstrap support values are annotated at each node with circles. Presence or absence of resistance is annotated at the outermost ring. Inner color strips display different CCs within each clade and the inner most ring displays the multilocus sequence type (ST). Plasmid mediated tetracycline resistance is distributed throughout the phylogeny along with resistance to fluoroquinolones. Clades 4 and 5 were the most common lineages recovered during the studied time frame.

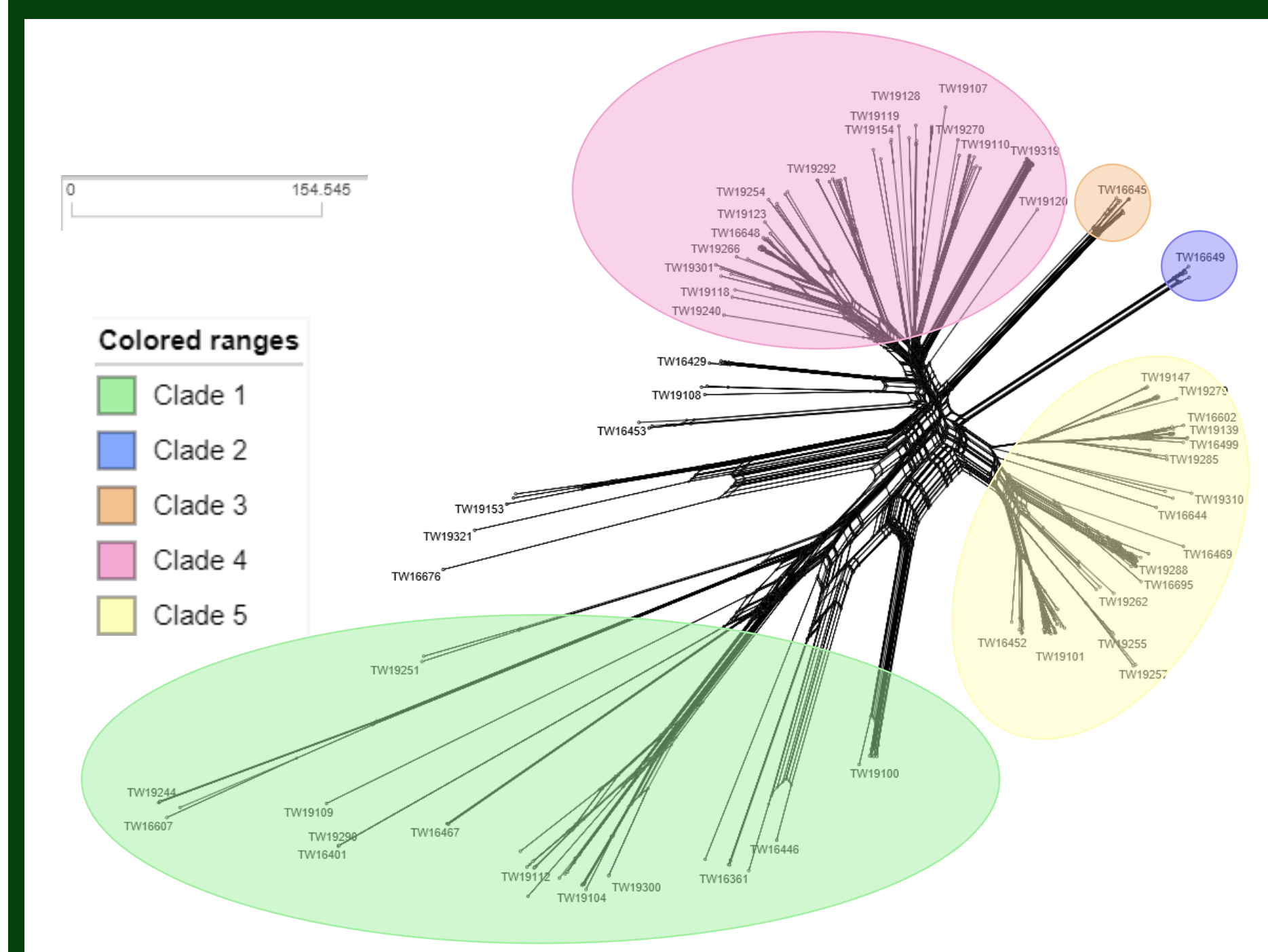
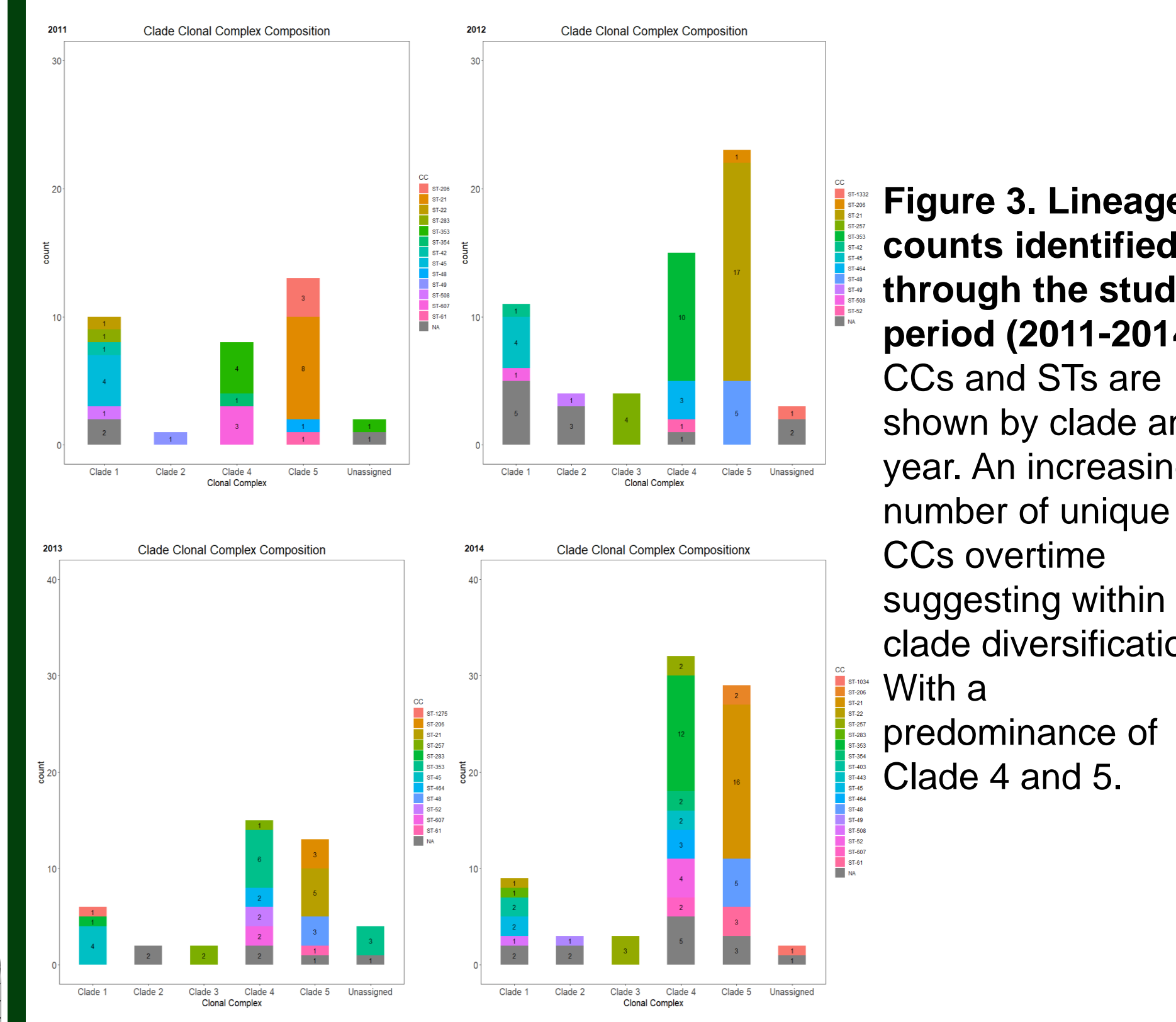


Figure 4. Neighbor-net analysis Neighbor net analysis confirmed the five clades identified in the maximum likelihood phylogeny. Significant recombination was observed among all 86 STs (pairwise homoplasy index $p < 0.00001$).

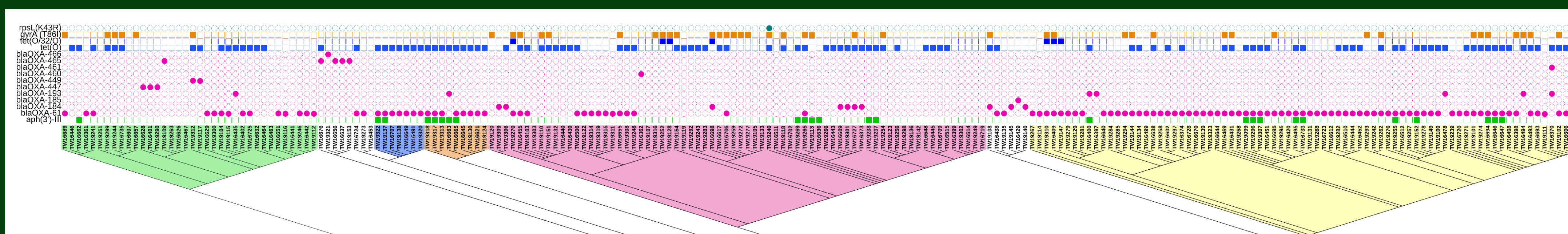


Figure 5. Identification of Resistance Determinants: Resistance genes and point mutations identified through Resfinder and Pointfinder (left) are shown within the "core" genome maximum likelihood phylogeny by clade. Susceptibility phenotypes were not confirmed for strains with β -lactamase genes and streptomycin mutations (circles), but resistance to aminoglycosides, tetracycline, fluoroquinolones (squares) was determined and are indicated by different colors

CONCLUSIONS

- Core genome MLST defined a "core" genome that includes 357 soft-core genes, 615 "core" and 1158 shell genes as well as 6640 cloud genes with a total of 8781 loci identified
- This cg-MLST approach utilizing 615 genes separated strains into five distinct clades with the maximum likelihood phylogenetic reconstruction
- Most strains were classified as clade 5 ($n=78$, 38.6%) and Clade 4 ($n=70$, 32.7%)
- Clade 2 and Clade 3 comprise two unique clusters of STs including ST467 ($n=3$), and ST922 ($n=7$)
- All but two clonal complexes (CCs) were restricted to unique clades. CC ST257 spanned Clades 3 and 4, while CC/ST353 was in Clade 4. Four strains comprising ST1036 ($n=1$) and ST1838 ($n=3$) did not cluster into a specific clade
- This finding suggests that cg-MLST is better able to detect the diversity of *C. jejuni* isolates than conventional MLST, which is important for epidemiological analyses
- The host-specialized lineage, ST353 ($n=18$), was the most common lineage followed by ST982 ($n=16$), ST50 ($n=11$) and the generalist lineage, ST45 ($n=11$)
- These data suggest that chicken (ST353) and ruminants (ST982, ST50) may be important reservoirs for *C. jejuni* infections in Michigan^{4,5}
- Neighbor-net analysis of the 214 *C. jejuni* isolates clearly separated the five clades and detected significant evidence of recombination
- Strain diversification was observed within CCs over time, particularly in the predominant Clades 4 and Clade 5
- The β -lactamase gene, *blaOXA-605*, was the most common resistance gene identified (58.8%: $n=125$) followed by *tet(O)* (56.0%: $n=121$), which highlights the importance of HGT for the distribution of these determinates as the genes are distributed throughout the phylogeny and across clades

FUTURE DIRECTIONS

- Genomic analysis of virulence genes, *fla* and LOS typing, assess congruence of phenotypic and genotypic data
- Comparison of network/minimum spanning trees will enable identification of clustered cases
- Epidemiological analysis to identify factors associated with each clade utilizing data from the Michigan Disease Surveillance System

ACKNOWLEDGMENTS

This work was supported by the NIH (U19AI090872) and MSU. We thank the four participating hospitals for help with specimen collection and Dr. James Rudrik, Ben Hutton and Jason Wholehan from the MDHHS for processing specimens. Attendance of this conference was made possible by the Thomas Whittam Travel Award. Additional support was provided through the Michigan State College of Osteopathic Medicine, College of Natural Science, the Ronald M. and Sharon Rogowski Fellowship and the MSU Foundation.

REFERENCES

- CDC. 2013. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2010.
- Foxman B, Zhang L, Koopman JS, Manning SD, Marrs CF. Choosing an appropriate bacterial typing technique for epidemiologic studies. 2005; 8:1-8.
- Besser JM, Carleton HA, Trees E, et al. Interpretation of Whole-Genome Sequencing for Enteric Disease Surveillance and Outbreak Investigation.
- Sheppard SK, Didelot X, Meric G, et al. Genome-wide association study identifies vitamin B5 biosynthesis as a host specificity factor in *Campylobacter*. Proc Natl Acad Sci U S A 2013; 110:11923-11927. Available at: <http://pubmlst.org/campylobacter/>. Accessed 17 September 2020.
- Cha W, Mosci RE, Wengert SL, et al. Comparing the genetic diversity and antimicrobial resistance profiles of *Campylobacter jejuni* recovered from cattle and humans. Front Microbiol 2017; 8:1-13.