

Genomic Structure and Diversity of Plasmids in *Campylobacter coli* and *Campylobacter jejuni*

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Abstract

Background: Emergence of antibiotic resistant *Campylobacter* among food producing animals and humans is a major public health concern. Plasmids have contributed greatly to the rapid dissemination of antimicrobial resistance (AMR) among enteric pathogens. **Purpose:** We investigated the genomic structure and diversity of plasmids harbored by *Campylobacter jejuni* and *C. coli* to gain knowledge of plasmid biology and the plasmid association with resistance and virulence. **Methods:** We selected 91 *C. jejuni* and *C. coli* by AMR resistance profiles. Isolates were sequenced with PacBio, Oxford nanopore and Illumina technologies. Sequences were assembled in GalaxyTrakr, using the FLYE assembler for long read assemblies and Unicycler for hybrid assemblies. Plasmid sequences were analyzed using the pangenome of coding and intergenic regions from *Campylobacter* plasmids, constructed with Roary and Piggy. Plasmids from the 91 *Campylobacter* isolates were grouped through hierarchical clustering of loci in R. The resistance genotypes for chromosomes and plasmids were identified separately using NCBI AMRFinderPlus. **Results:** Ninety-nine plasmids (1.7kb-155kb) were detected from 91 *Campylobacter* isolates. Twenty-two isolates didn't carry any plasmid, 50 isolates contained a single plasmid, 12 isolates had 2 plasmids, 3 isolates had 3 plasmids and 4 isolates had 4 plasmids. Pangenomic analysis classified these 99 plasmids into 10 groups. Group 1 represented 17 pVir plasmids containing the *virB11* virulence gene. Five pVir plasmids carried the *tet(O)* AMR gene. Group 2 contained 22 plasmids with the pTet plasmid backbone, though 3 plasmids lacked the *tet(O)* gene. Further, Group 2 plasmids also encoded for the virulence genes *virB2*, *virB4*, *virB8*, *virB9* and *virD4*. Group 3 contained 19 uncharacterized plasmids and all 11 instances of *aph(3'')-VIIa* in our dataset were found only in this group. Group 4 had 21 plasmids and was characterized by the presence of *virB4* and *virC1*. The remaining 20 plasmids were categorized into 6 groups that did not contain any AMR or virulence genes. **Conclusion:** Our study indicates *Campylobacter spp.* carry genetically diverse plasmids. Many of these were hybrid AMR-virulence plasmids that have the potential to spread among food producing animals and humans. This study also provides a tool for subtyping *Campylobacter* plasmids which is important to support outbreak investigations and study the epidemiology of plasmid biology associated with resistance and virulence as well as the source of isolates.

Introduction

Campylobacter is the major bacterial foodborne pathogen worldwide and more than one million cases yearly are reported in the United States alone. The global emergence of antimicrobial resistance in foodborne pathogens has become a major public health concern. The CDC has classified drug-resistant *Campylobacter* as a serious antibiotic resistance threat in the U.S. Plasmids have contributed greatly to the rapid dissemination of antimicrobial resistance among enteric pathogens. The acquisition of plasmids carrying antimicrobial resistance or virulence genes can significantly alter the prevalence of multidrug resistance (MDR) or virulence bacterial clones. Therefore, the study of plasmid type and genomic structure are very important to understand the molecular epidemiology of MDR bacterial infections. The aim of this study was to close the genomes of MDR *C. jejuni* and *C. coli*; and investigate the diversity and genomic structure of plasmid and use the coding regions and the intergenic regions (pangenomes) to develop a subtyping scheme for *Campylobacter* plasmids. Knowing plasmid diversity and the subtype will help us to understand antimicrobial resistance development, dissemination and co-selection pressure from different food animal production environments.

Materials and Methods

Bacterial strains:

A total of 91 *Campylobacter* isolates recovered from cattle, retail meats, companion animals (puppies), and humans from 2017-2021 were collected through NARMS culture collection. Isolates were grown on sheep blood agar plates (Thermo Fisher Scientific, Remel, Lenexa, KS) at 42°C under microaerobic conditions for antibiotic susceptibility test (AST) and genomic DNA extraction for sequencing.

Antimicrobial Susceptibility Testing (AST):

Antimicrobial Susceptibility testing was performed by broth micro dilution using the NARMS *Campylobacter* panel (catalog#: CAMPY, Thermo Fisher Scientific, Trek Diagnostics, Cleveland, OH).

Genome DNA extraction, sequencing, assembly:

Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega). To close the genomes of 82 *Campylobacter* Isolates long read sequencing was performed on Oxford Nanopore Technologies platform using MinION Sequencing Device MIN-101B, Flow Cell R9.4.1 version and Rapid barcoding Kit SQK-RBK004 (Oxford Nanopore, UK). Short read sequencing was done on MiSeq platform using v3 reagent kits (Illumina, San Diego, CA, USA). Genomes of nine *Campylobacter isolates* were closed by long read sequencing using Pacific Biosciences (PacBio) RS II Sequencer (PacBio®, Menlo Park, CA, USA).

Nanopore sequences were assembled in GalaxyTrakr, using the FLYE assembler for long read assemblies and Unicycler for hybrid assemblies when long reads were combined with Illumina short reads. Continuous-long-reads from PacBio were assembled using PacBio Hierarchical Genome Assembly Process (HGAP3.0)

Analysis of plasmid sequences:

Genome annotation was done using PROKKA. Roary and Piggy pipelines were then used to generate pangenome of coding and intergenic regions from *Campylobacter* plasmids. Finally, *Campylobacter* plasmid groups were determined through hierarchical clustering in R.

How this study supports FDA's mission

- In this study, we investigated the diversity of *Campylobacter* plasmids and developed a subtyping method that could support *Campylobacter spp* outbreak investigation.

- This study will help us understand the plasmid biology associated with AMR development, dissemination, and co-selection pressure from various food animal production environments, which is important for the food industry and healthcare professionals to develop strategies to stop the spread of antibiotic-resistant organisms.

- Our data will help FDA to assess the threat of antibiotic resistance and virulence of *Campylobacter spp.* to humans and animals.

Results and Discussion

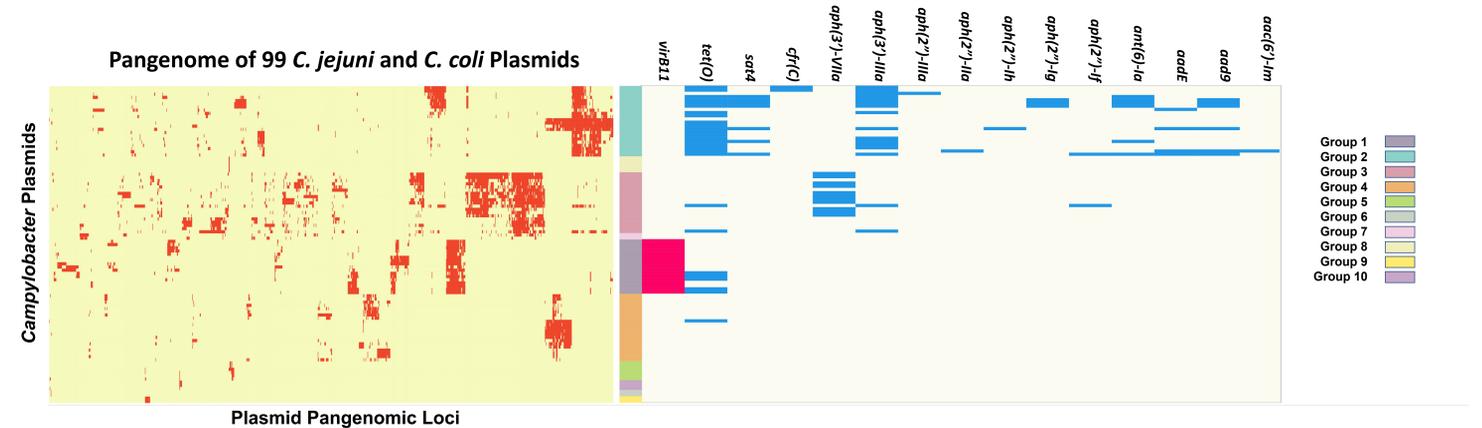


Figure 1. Pangenome of the 99 plasmids recovered from *Campylobacter* isolates. Plasmid loci contain both the coding regions and intergenic regions. Orange color indicates the presence of a virulence gene **VirB11** while the blue color indicates the presence of a plasmid locus while light yellow indicates absence

Figure 2. Metadata for the 99 *Campylobacter* plasmids. Order of the plasmids corresponds to the order in Figure 1. Red color indicates the presence of a virulence gene **VirB11** while the blue color indicates the presence of an antimicrobial resistance gene.

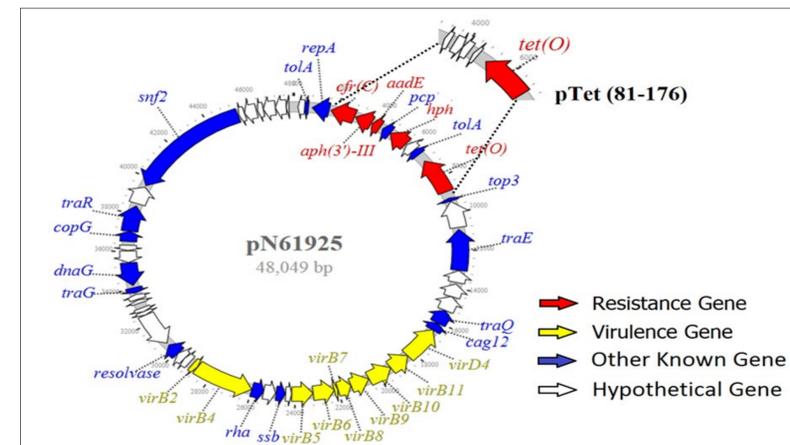


Figure 3: Structure of the hybrid plasmid from plasmid group 2

Plasmid Group	(n)	Plasmid type	virB11	virB11 and tet(O)	tet(O)	T4SS without tet(O)	T6SS	aph(3'')-IIIa
1	17	pVir	12	5	0	0	0	0
2	22	pTet	19	0	3	0	0	0
3	19	None	0	0	0	0	19	11
4	21	pT4SS	0	0	0	21	0	0
5-10	20	None	0	0	0	0	0	0

Table 1: Plasmid Group based on the presence of genes

Conclusions

- Campylobacter spp.* carry genetically diverse plasmids
- Ninety-nine plasmids were identified in sixty nine isolates while twenty two other isolates did not carry any plasmid. 80% (n=99) of these plasmids are highly diverse.
- These diverse groups of plasmids were classified into four major groups which include 48.7% conjugative (n=41) plasmids carrying AMR genes, and virulence gene, some of them being hybrid plasmids, with potentials to spread among food producing animals and humans.
- Our study provides a tool to subtype *Campylobacter* plasmids which will help support outbreak investigation and study epidemiology of *Campylobacter* plasmid biology.

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