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Campylobacter jejuni and its Effect on American Poultry

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Abstract

Campylobacter jejuni (*C. jejuni*) is a pathogen responsible for the majority of worldwide enteritis. In certain cases, post-infection diseases may result in full-body paralysis or even death within the host. While *C. jejuni's* natural hosts are avian species, it can infect humans through contaminated meats, dairy products, or water sources. Using whole genome sequences from national databases, an annotated phylogenic tree was created from *C. jejuni* and analyzed through visual diagrams. These trees revealed the scattered nature of the *C. jejuni* infections; while there were multiple clusters of closely related strains, clusters were often very distant from one another in terms of relatedness. They also showed multiple instances of *C. jejuni* infecting different species of hosts between closely related strains, which may potentially lead to its rapid spread in geographic regions and a potential increase in the number of species impacted by its spread. Additionally, it revealed a correlation between antimicrobial resistance (AMR) genes in chickens and the US geolocation. These results may be explained through *C. jejuni's* ability to exchange genes and its ability to adapt quickly to its environment. As *C. jejuni* still remains at large in the common age, more must be understood about the nature of its infections. Thus, this study aims to explore possible correlations between host species and geographic locations of *C. jejuni* bacterial strains.

Keywords: Campylobacter, Antimicrobial Resistance Genes, Phylogeny

1. Introduction

Campylobacter jejuni is one of the leading causes of bacterial enteritis worldwide (Altekruse et al., n.d.). This rod-shaped gram-negative bacterium has a relatively small genome of only 1,743,985 bp (G+C content of 30.3%) (He et al., 2020) whereas the common bacterial genome reaches about 5 million bp (Land et al., 2015), indicating that there are less proteins being created through its core genome. As a result, this bacterium may rely more on plasmids in order to function and to infect its hosts. *C. jejuni* can naturally take up genetic material, both plasmids and chromosomal DNA, from its surroundings (Young et al., 2007). These characteristics allow for each strain of *C. jejuni* to differ greatly from one another through their highly variable surface proteins (Young et al., 2007). This bacterium can exchange large amounts of genetic information between individuals and other species of *Campylobacter*, mainly *C. coli*, through horizontal gene transfer (Sheppard & Maiden, 2015). All of these characteristics result in a highly adaptable bacterium that may differ greatly, even among closely related strains of the same species.

C. jejuni is a waterborne and foodborne pathogen whose natural hosts are chickens and other avian organisms (Young et al., 2007). The bacterium only results in benign infections in chicken hosts whereas in humans results in severe inflammation and enteritis (Pielsticker et al., 2012). Upon infection, human hosts experience up to two weeks of inflammation, abdominal pain, fever, and diarrhea (Young et al., 2007). Within the developed world, *C. jejuni* infections are characterized by bloody diarrhea with mucus and watery diarrhea within the undeveloped world (Peterson, 1994). Children are more commonly infected in the undeveloped world, leading to lower infection rates in addition to reduced symptom severity as adults due to the early infection acting as a vaccine for later infections (Young

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et al., 2007) (Kaakoush et al., 2015). Post-infection symptoms may be more detrimental to the host compared to symptoms that occur during the period of infection. *C. jejuni* is believed to cause Guillain-Barre syndrome, and Reiter Syndrome (Peterson, 1994). Guillain-Barre syndrome is an autoimmune disorder in which the human immune system mistakes the nerves in the body as a pathogen. Due to the similarities in surface proteins in infecting *C. jejuni* bacteria and its host's neurons, antibodies that are created to target *C. jejuni* may target the human body's own nervous tissue gangliosides instead (Nyati & Nyati, 2013). As a result, the immune system degrades the myelin sheath and axons of the neurons targeted. Guillain-Barre Syndrome then ensues, beginning from the weakness and tingling in feet and hands to the paralysis of the whole body (Peterson, 1994). The apex of this infection is a full paralytic neuropathy of the host and can be fatal. Full recovery may take up to a few years, although many patients are typically able to walk within six months (Nyati & Nyati, 2013). Reiter Syndrome is a specific type of arthritis characterized by joint pain, stiffness, and inflammation within the eyes, knees, ankles, and feet. In some cases, this may result in blurred vision in the human host (Peterson, 1994).

Infections occur within the gastrointestinal tract of the host. *C. jejuni* in chickens, its preferred host, can replicate to high numbers such as 10^{10} units per gram of infected tissue. Infections may pass from host-to-host through the fecal-oral route, or through infected water supplies (Young et al., 2007). In infected waters, *C. jejuni* may form biofilms with amoebae and other protozoans (Snelling et al., 2005). Humans are infected through the consumption of contaminated meats, unpasteurized milk, or water from contaminated sources (Peterson, 1994). Upon infection of the human body, the corkscrew-like movement of *C. jejuni* enables it to circulate through the mucus layer of the gastrointestinal tract and infect the gastrointestinal epithelial layer (Young et al., 2007). *C. jejuni* in humans may also release a cytolethal-distending toxin (CDT) that triggers cell-cycle arrest and subsequently death in the host cell (Whitehouse et al., 1998).

C. jejuni, although benign in chicken hosts, causes harmful and occasionally lethal infections in humans (Gripp et al., 2011). Thus, this study further investigates the evolutionary patterns regarding the bacterium to identify possible correlations.

2. Materials and Methods

C. jejuni isolates from the Assembly (Kitts et al., 2016) and Nucleotide (S, 2012) databases from the National Center for Biological Information (NCBI)'s National Library of Medicine were filtered for entries with complete Refseq genomes (O'Leary et al., 2016). The Refseq database was used in order to maintain higher quality genomes as the entries in the database are reviewed and validated. The entries were then sorted by relevance and the first forty entries were collected. Metadata for these genomes were collected from the BioSample (Barrett et al., 2012) database on NCBI. These isolates' host species range from human, chicken, bovine, mouse, turkey, and American Black Bear. The origin of collection of each genome spans across multiple different countries including Thailand, Taiwan, USA, Belgium, United Kingdom, Sweden, and Finland.

The collected genome assemblies were annotated using the bakta software (Schwengers et al., 2021). Bakta cross-references multiple public databases in order to create a precise and thorough annotation of the genomes. A core alignment and a pangenome was calculated through panaroo. (Tonkin-Hill et al., 2020). Using this core genome alignment, create a maximum likelihood tree was created using IQ-Tree (Chernomor et al., 2016). Then, pathogenwatch was used to find antimicrobial resistant (AMR) genes in the collected isolates (Argimón et al., 2021). Microreact was used to visualize the phylogenic tree (Argimón et al., 2016).

Table 1. Table of all the isolates that have been shown by Pathogenwatch to contain the AMR gene T861. This gene provides resistance against Ciprofloxacin, a fluoroquinolone used to treat gastrointestinal diseases (American Society of Health-System Pharmacists, 2024). It is clear that a large portion of these strains have been isolated from human and avian hosts.

Strain	Host	Geo Location
BDS5	Farm	USA
Genome 1	Chicken	Sweden
ZP3204	Chicken	USA
CS19	Chicken	South Korea
IF1100	Chicken	USA
R19.1007	Human	Taiwan
CJ018CCUA	Human	Finland
R18.1301	Human	Taiwan
CJ071CC464	Human	Finland
YQ2210	Turkey	USA
CFSAN054107	Unknown	Thailand
AR-0414	Unknown	Unknown

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3. Results

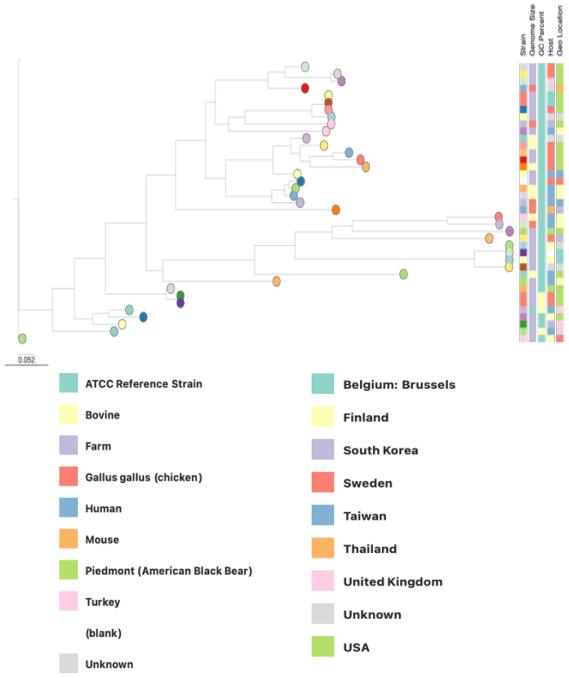

Figure 1. The ML phylogenetic tree as visualized by Microreact, along with the corresponding isolate metadata. The GC content percentage and genome size does not vary greatly, with variations in GC content being displayed only in older lineages.

Figure 2. Subset of the ML tree showing *C. jejuni* samples isolated from chickens (Gallus gallus). Branches with colored dots still remaining on their ends indicate *C. jejuni* strains that have chicken hosts. Most of the genomes collected are found in clusters and have been sampled in the US.

3 Discussion

In Figure 1 and in Figure 2, the length of each branch represents the distance of each strain from its most recent ancestor. In Figure 1, there are many instances of rapid evolution evidenced by short branch lengths of isolates clustering together. Some samples have branches that are very long and separate from the other strains, which reveal that the strain is very distant from its most recent relative. Since it is unlikely for a bacterial strain to have not evolved for many generations, it is possible that the strain is an outlier. Within each cluster, there are multiple different host species between closely related strains.

In Figure 2, the vast majority of the isolates are shown as a part of a cluster representing rapid evolution between *C. jejuni* strains. Bacterium with AMR genes are often able to proliferate and evolve at a much higher rate, and thus it is notable that these strains largely occur in clusters (Davies & Davies, 2010). Additionally, the majority of these strains have an American geographical location of origin.

Table 1 isolates the strains with AMR genes. This table displays a strong association between avian hosts and American geographical location of origin when reviewing only the resistant strains.

C. jejuni is not specifically adapted to one main host, as evidenced by the multiple different hosts and the tendency for *C. jejuni* to interchange hosts. This can be caused by this bacterium's ability to survive without a host in aquatic mediums by forming biofilms with unicellular organisms. *C. jejuni* can therefore easily transfer from one host to another if a water supply is contaminated.

One possible reason for the instances of rapid evolution and separated, longer branches is the sparsity of the collected data. A dataset with more strains may provide a more accurate depiction of possible outbreaks. Additionally, the possible outliers may be revealed to have a much more recent ancestor once the lack of strains are addressed. There is a possibility that the results created from this data may not accurately be representative to all of American *C.jejuni*

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bacterium due to the small sample size. Future research should reevaluate these results using a sample size of hundreds of *C.jejuni* strains to ensure better accuracy.

Another possible reason is *C. jejuni*'s ability to exchange genes with other bacterial cells of the same species as well as other Campylobacter species in horizontal gene transfer. During the process, certain strains of *C. jejuni* can rapidly become genetically different than the other strains. As such, the outliers in the phylogenetic tree (Figure 1) could be the result of increased horizontal gene transfer events, as they differ greatly from all the other strains.

Other researchers have found similar challenges with analyzing *C. jejuni* strains due to its ability to recombine, even with other closely related species in the same genus. A study examining the natural transformation of *C. jejuni* found that using PCR on hybrid strains results in ambiguous information being collected while MLST typing is impaired (Golz et al., 2020). This could explain the presence of outlier strains in the maximum likelihood phylogeny, that showed longer, and deeper branch lengths compared to the rest of the tree. The outlier samples may have also been mistakenly labeled as *C. jejuni*.

A study analyzing the metabolism of *C. jejuni* discovered that this bacterium is able to utilize multiple different amino acids for metabolic purposes (Hofreuter et al., 2008). Another study analyzing host-specific phenotyping for *C. jejuni* found little evidence for host adaptation and instead found high genetic flexibility for a changing environment (Gripp et al., 2011). These factors show that *C. jejuni* is a bacterium that is able to survive in multiple different hosts, in agreement with the multiple hosts jumps that can be inferred from the core genome phylogeny presented in this report.

A study has shown that the use of fluroquinolones to temporarily treat domestic chickens increases the amount of fluoroquinolone resistant strains of *C. jejuni* after continued usage. It also explains that the decrease in fluroquinolone usage will decrease the likelihood of AMR genes to appear in *C. jejuni* strains (Iovine & Blaser, 2004). *C. jejuni* strains with fluoroquinolone-resistant genes has sharply increased within the past 20 years in America (Nachamkin et al., 2002), which one study suggests has arrived from the treatment of domestic chickens with fluroquinolones (Smith et al., 1999). These studies support my results of antimicrobial resistant strains appearing in American chickens. However, there is not sufficient data to associate chicken-associated isolates with a US geographical origin. Furthermore, more data is required to accurately associate treatment of chickens with fluoroquinolones to the development of AMR genes in *C. jejuni*.

4 Conclusion

The ability of *C. jejuni* to adapt quickly in a changing environment allows for it to rapidly transfer host species between closely related strains. Large gaps between clusters of related strains and strain lineages with little evolution can be attributed with *C. jejuni's* ability to recombine frequently and exchange genes with other strains and species of Campylobacter.

The data shows an association between chicken outbreaks with AMR and geolocation as many of the chicken-host strains have an American origin and vice versa. One possible reason for this association is the treatment of domestic chickens with fluroquinolone antibiotics in America. Horizontal gene transfer, which is common amongst *C. jejuni* isolates, is a major factor in the development of resistance and could have been an underlying factor affecting these results. More research must be conducted to conclude causation for this relationship.

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References

Altekruse, S. F. et al. (n.d.). Campylobacter jejuni—An Emerging Foodborne Pathogen—Volume 5, Number 1—February 1999—Emerging Infectious Diseases journal—CDC. http://doi.org/10.3201/eid0501.990104

American Society of Health-System Pharmacists. (2024, May 10). Ciprofloxacin Monograph for Professionals. Retrieved October 13, 2024, from https://www.drugs.com/monograph/ciprofloxacin.html

Argimón, S. et al. (2016). Microreact: Visualizing and sharing data for genomic epidemiology and phylogeography. *Microbial Genomics*, *2*(11), e000093. http://doi.org/10.1099/mgen.0.000093

Argimón, S. et al. (2021). A global resource for genomic predictions of antimicrobial resistance and surveillance of Salmonella Typhi at pathogenwatch. *Nature Communications*, *12*(1), 2879. http://doi.org/10.1038/s41467-021-23091-2

Barrett, T. et al. (2012). BioProject and BioSample databases at NCBI: Facilitating capture and organization of metadata. *Nucleic Acids Research*, *40*(Database issue), D57-63. http://doi.org/10.1093/nar/gkr1163

Chernomor, O., von Haeseler, A., & Minh, B. Q. (2016). Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Systematic Biology*, *65*(6), 997–1008. http://doi.org/10.1093/sysbio/syw037

Davies, J., & Davies, D. (2010). Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews : MMBR*, *74*(3), 417–433. http://doi.org/10.1128/MMBR.00016-10

Golz, J. C. et al. (2020). Whole genome sequencing reveals extended natural transformation in Campylobacter impacting diagnostics and the pathogens adaptive potential. *Scientific Reports*, *10*(1), 3686. http://doi.org/10.1038/s41598-020-60320-y

Gripp, E. et al. (2011). Closely related Campylobacter jejuni strains from different sources reveal a generalist rather than a specialist lifestyle. *BMC Genomics*, *12*(1), 584. http://doi.org/10.1186/1471-2164-12-584

He, Y., Reed, S., & Strobaugh, T. P. (2020). Complete Genome Sequence and Annotation of Campylobacter jejuni YH003, Isolated from Retail Chicken. *Microbiology Resource Announcements*, *9*(4), e01307-19. http://doi.org/10.1128/MRA.01307-19

Hofreuter, D., Novik, V., & Galán, J. E. (2008). Metabolic diversity in Campylobacter jejuni enhances specific tissue colonization. *Cell Host & Microbe*, *4*(5), 425–433. http://doi.org/10.1016/j.chom.2008.10.002

Iovine, N. M., & Blaser, M. J. (2004). Antibiotics in Animal Feed and Spread of Resistant Campylobacter from Poultry to Humans. *Emerging Infectious Diseases*, *10*(6), 1158–1189. http://doi.org/10.3201/eid1006.040403

Kaakoush, N. O. et al. (2015). Global Epidemiology of Campylobacter Infection. *Clinical Microbiology Reviews*. http://doi.org/10.1128/cmr.00006-15

Kitts, P. A. et al. (2016). Assembly: A resource for assembled genomes at NCBI. *Nucleic Acids Research*, *44*(Database issue), D73–D80. http://doi.org/10.1093/nar/gkv1226

Land, M. et al. (2015). Insights from 20 years of bacterial genome sequencing. *Functional & Integrative Genomics*, *15*(2), 141–161. http://doi.org/10.1007/s10142-015-0433-4
Nachamkin, I., Ung, H., & Li, M. (2002). Increasing Fluoroquinolone Resistance in Campylobacter jejuni, Pennsylvania, USA,1982–200111. *Emerging Infectious Diseases*, *8*(12), 1501–1503. http://doi.org/10.3201/eid0812.020115

Nyati, K. K., & Nyati, R. (2013). Role of Campylobacter jejuni infection in the pathogenesis of Guillain-Barré syndrome: An update. *BioMed Research International*, *2013*, 852195. http://doi.org/10.1155/2013/852195

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ISSN 2688-3651 [online]

O'Leary, N. A. et al. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733-745. http://doi.org/10.1093/nar/gkv1189

Peterson, M. C. (1994). Clinical aspects of Campylobacter jejuni infections in adults. *The Western Journal of Medicine*, *161*(2), 148–152.

Pielsticker, C., Glünder, G., & Rautenschlein, S. (2012). Colonization properties of Campylobacter jejuni in chickens. *European Journal of Microbiology & Immunology*, *2*(1), 61–65. http://doi.org/10.1556/EuJMI.2.2012.1.9

S, F. (2012). The NCBI Taxonomy database. *Nucleic Acids Research*, *40*(Database issue). http://doi.org/10.1093/nar/gkr1178

Schwengers, O. et al. (2021). Bakta: Rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microbial Genomics*, *7*(11), 000685. http://doi.org/10.1099/mgen.0.000685

Sheppard, S. K., & Maiden, M. C. J. (2015). The Evolution of Campylobacter jejuni and Campylobacter coli. *Cold Spring Harbor Perspectives in Biology*, *7*(8), a018119. http://doi.org/10.1101/cshperspect.a018119

Smith, K. E. et al. (1999). Quinolone-resistant Campylobacter jejuni infections in Minnesota, 1992-1998. Investigation Team. *The New England Journal of Medicine*, *340*(20), 1525–1532. http://doi.org/10.1056/NEJM199905203402001

Snelling, W. J. et al. (2005). Survival of Campylobacter jejuni in Waterborne Protozoa. *Applied and Environmental Microbiology*, *71*(9), 5560–5571. http://doi.org/10.1128/AEM.71.9.5560-5571.2005

Tonkin-Hill, G. et al. (2020). Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biology*, *21*(1), 180. http://doi.org/10.1186/s13059-020-02090-4

Whitehouse, C. A. et al. (1998). Campylobacter jejuni cytolethal distending toxin causes a G2-phase cell cycle block. *Infection and Immunity*, *66*(5), 1934–1940. http://doi.org/10.1128/IAI.66.5.1934-1940.1998

Young, K. T., Davis, L. M., & DiRita, V. J. (2007). Campylobacter jejuni: Molecular biology and pathogenesis. *Nature Reviews Microbiology*, *5*(9), 665–679. http://doi.org/10.1038/nrmicro1718