Multi-locus sequence typing (MLST) of \textit{C. jejuni} infections in the United States

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Setting the stage

Campylobacter: a major health threat

One of the most common foodborne pathogens in the United States based on 2011 estimates*:
- # illnesses: 4th
- # hospitalizations: 3rd
- # deaths: 5th

*http://www.cdc.gov/foodborneburden
Foodborne Diseases Active Surveillance Network (FoodNet)

- Established in 1996
- Track important foodborne illnesses and generate information that provides a foundation for food safety policy and prevention efforts
- Collaboration among the Centers for Disease Control and Prevention (CDC), ten participating state health departments, the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS), and the Food and Drug Administration (FDA)
FoodNet Sites 2011

47 million (~15% of U.S. population)
FIGURE 1. Estimated percentage change in incidence of laboratory-confirmed bacterial and parasitic infections in 2012 compared with average annual incidence during 2006–2008, by pathogen — Foodborne Diseases Active Surveillance Network, United States

- What are the most common C. jejuni strains/lineages circulating among human infections?
- What are the sources of infection?

Adapted from: Mortality and Morbidity Weekly Report (MMWR), April 19, 2013 / 62(15);283-287
Increased fluoroquinolone resistance observed in *C. jejuni* human infections in the US

- Increased prevalence of fluoroquinolone-resistant *C. jejuni* among human isolates in the past decade have been well-documented ([http://www.cdc.gov/narms/](http://www.cdc.gov/narms/))

- National Antimicrobial Resistance Monitoring System (NARMS)
  - Monitors antimicrobial resistance among *Campylobacter* isolated from humans (CDC), retail meats (FDA) and food animals (USDA) in the US
  - Isolates tested for nine antimicrobials:
    - Azithromycin, Erythromycin, Gentamycin, Clindamycin, Florfenicol, Telithromycin, Ciprofloxacin, Nalidixic Acid and Tetracycline

- Sarafloxacin and enrofloxacin, introduced in 1995 and 1996 as poultry medicine, were withdrawn in 2005 by FDA
Trend in ciprofloxacin resistance among *C. jejuni* human infections from NARMS 1997-2010

- Are specific clonal lineages associated with resistance?
- What are the relative proportion of resistant isolates within these lineages?
Our *Campylobacter* source attribution project

- **AIM:** To generate and integrate human data (antimicrobial, epidemiological and molecular typing data) into different source attribution models with U.S. and international source data

**Data types**
- Demographic
- Clinical
- Exposure
- Antimicrobial

**Human isolates**
- Sporadic infection and outbreaks

**Source isolates**
- Animals, retail meat, wildlife, environmental

**Lab data types**
- MLST
- Whole genome sequence (WGS)?

**Island model, Dutch model**
- Adapted Danish model

**Adapted Island model or others...**

**Data types**
- Geographical Sampling scheme
- Isolation site
Our *Campylobacter* source attribution project

- **Sporadic *C. jejuni* human isolates from:**
  - 789 isolates were characterized by MLST
    - Available FoodNet sites in 1998 (n=303)
    - All FoodNet sites and WY in 2008 (n=483)
  - Antimicrobial susceptibility data (NARMS)
  - Associated epidemiological and exposure data also available

- **Outbreak *C. jejuni* human isolates from:**
  - A historical collection at CDC and prospective outbreaks have been characterized by MLST (60+ outbreaks)

- **Attributing our dataset to sources**
  - Dutch model
  - Adapted Danish model
  - Island model, adapted Island model
Significant decline in *Campylobacter* infections in New Zealand

Decline in human cases after intervention

Adapted from Baker et al., 2007
Main sources of sporadic infection identified

(I) Danish model, (II) Modified Hald model, (III) Island model

Adapted from French et al., 2010
Models 101: Dutch, Danish and Island models

• **Dutch model**
  – Compares the number of human cases caused by different subtypes with their prevalence in each source.

• **Modified Danish model**
  – Similar to the Dutch model, compares the number of human cases caused by different subtypes with their prevalence in each source, but with additional weighting explicitly added for each parameter.

• **Island model**
  – Fundamentally different from Dutch and Danish models: population genetics based
  – Modeling the genealogy of isolates based evolutionary processes of mutation and recombination, using allelic information to take account into the relatedness of subtypes
  – Allows for novel combination of alleles in human isolates not observed in source populations.
Molecular Evidence Attributes the Source of a *Campylobacter* Outbreak Associated with the Consumption of Raw Peas to Wild Birds


15/55 human isolates (4 PFGE patterns) were indistinguishable from isolates from 4 environmental specimens (2 pea, 2 crane feces) by PFGE.

Can we attribute the other human cases who did not match by PFGE also to the cranes?
Genetic relatedness of *C. jejuni* strains using MLST from the Alaska outbreak and the MLST database.

- **Red** = Crane isolates
- **Orange** = Human outbreak isolates
- **Light Blue** = Wild bird isolates
- **Dark Blue** = Environmental isolates
- **Green** = Human isolates
- **Pink** = Chicken isolates
New Possibilities from using WGS in Source Attribution Analysis

- Ability to further differentiate genotypes provides the level of resolution needed to visualize within-strain differences (e.g. evolution of antimicrobial resistance)
- Differences in gene content between strains may show correlation for host-specificity and antimicrobial resistance
- Allows direct comparison across *Campylobacter* species
Next: WGS short-term goal

- **MLST vs WGS**
  - Using WGS data from all representative isolates from sources
  - Further differentiation of major clonal lineages
  - Re-analyze antimicrobial and epidemiological data with full genome resolution

- **Gene by gene analysis**
  - Differences in pathogenicity, host-specificity and antimicrobial resistance between strains may correlate with gene content

- **Refine models with WGS data**
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Questions?

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