A genomic and modelling approach to understanding the evolution, source and transmission of a recently emerged antimicrobial resistant lineage of Campylobacter


OHA 2020
New Zealand: Food and waterborne diseases high on the list of notifiable diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacteriosis</td>
<td>6202</td>
</tr>
<tr>
<td>Measles</td>
<td>2213</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>1749</td>
</tr>
<tr>
<td>Pertussis</td>
<td>1206</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>1188</td>
</tr>
<tr>
<td>Yersiniosis</td>
<td>1186</td>
</tr>
<tr>
<td>Shiga toxin-producing <em>Escherichia coli</em> infection</td>
<td>1101</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>1035</td>
</tr>
<tr>
<td>Invasive pneumococcal disease</td>
<td>497</td>
</tr>
<tr>
<td>Acute gastroenteritis</td>
<td>489</td>
</tr>
</tbody>
</table>

Most zoonotic, many food and waterborne
Campylobacter in NZ: 1980-2006

Regulation of chicken contamination urgently needed to control New Zealand’s serious campylobacteriosis epidemic

Michael Baker, Nick Wilson, Rosemary Ikram, Steve Chambers, Phil Shoemack, Gregory Cook
Manawatū sentinel site 2005-2018

• ‘One health’ approach: human, animal and environment
• Identify Multilocus Sequence Types common to particular sources
• Modelling (reservoir attribution)
Manawatu sentinel site
2005-18
*Campylobacter* population structure:
according to 7-genes

![Graph showing the population structure of Campylobacter jejuni and C. coli with various samples categorized by source such as Human, Other, Poultry, Ruminants, and Water.](image)
Reservoir attribution
‘Asymmetric Island model’ (AIM)

- Population genetics / evolutionary modelling approach
- Used to find out source of human infections
- Flow into the human “island” from animal “islands”

Tracing the Source of Campylobacteriosis

Daniel J. Wilson¹nas, Edith Gabriel²mb, Andrew J. H. Leatherbarrow³, John Cheesbrough⁴, Steven Gee⁵, Eric Bolton⁶, Andrew Fox⁷,⁸, Paul Fearnhead¹, C. Anthony Hart⁶,⁷, Peter J. Diggle²
Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach

Mullner et al
Campylobacter in Poultry – Risk Management Strategy
2007 - 2010
Relationship between campylobacteriosis notifications and hospitalisations

Data Sources: ESR Ltd notification data; NZHIS hospitalisation data (filtered)
Marked Campylobacteriosis Decline after Interventions Aimed at Poultry, New Zealand

50% decline in notifications and hospitalisations
Est $50-70M saving per annum

Sears et al 2011, Emerging Infectious Diseases 17, 1007-15

Large waterborne outbreak
J Infect. 2020 Sep;81(3):390-395
Arrival of ST-6964 in 2014 and antibiotic resistance

Superbug found in chicken

Scientists are alarmed at the unprecedented discovery of a strain that resists drugs and has crossed into humans around NZ, writes Susan Edmunds.

A new superbug has been found in chickens, from three of New Zealand’s main meat poultry suppliers. A groundbreaking research reveals the new antibiotic-resistant strain of bacteria, which could make it hard to treat serious cases of infections.

Campylobacter occurs naturally in the gut of chickens but is the leading cause of food poisoning, with about 1000 cases reported in New Zealand each year. The antibiotic-resistant strain has been identified in humans in Massachusetts, Indiana and Wellington. A study by Nigel French of Massey University and US microbiologist Debbie Wallis, found three of the main poultry suppliers in the North Island were infected with the strain. A fourth was still waiting for test results. The pair would not name the companies.

This strain means two antibiotics – fluoroquinolones and trimethoprim – would fail in treating the infection, and

Which comes first?
The chicken or the campylobacter

1. Campylobacter enters the gut
2. Campylobacter spreads through the chicken
3. Campylobacter enters the people
4. Campylobacter spreads through the people

The chicken or the campylobacter infected by people.
Antibiotic resistant Campylobacter in NZ

Resistance in humans similarly low ~1-3% FQ

Very low by international standards
Low use of Abs in New Zealand poultry industry

<table>
<thead>
<tr>
<th>Drug</th>
<th>Disc (µg)</th>
<th>Res (%)</th>
<th>≤6</th>
<th>7-15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>≥28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>19</td>
<td>12</td>
<td>21</td>
<td>122</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Concentration of drug within the disc
* Percentage of isolates with zone sizes within the resistant category for that drug
Whole genome sequencing and Antimicrobial Resistance: Emergence of new strain: *Campylobacter jejuni* ST-6964

- **August 2014**: first two human cases of new *C. jejuni* ST-6964 detected in sentinel site
- Found in 4 poultry companies and ‘breeder’ (parent) flocks
- **Resistant to tetracycline and fluoroquinolones**
  - 0->37% in poultry
  - Sharp increase in AMR and ST 6964 in human cases across NZ (ESR study)
  - Chicken liver outbreak, Wellington
Whole genome sequencing showed AMR strain was new to New Zealand.
Key questions?

- How long has it been in NZ?
- How has it been transmitted between poultry companies?
- What has driven the emergence?
- What is the main source of human infection?
- How is it evolving?
- Genetic basis for resistance?

These could only be addressed by Whole Genome Sequencing and a One Health approach.

Fig. 2: Social network analysis of feed-related contacts in the New Zealand
Pacbio and Illumina sequencing of 230 *C. jejuni* ST-6964 isolates from poultry farms and human cases

NeighborNet of wgMLST allelic profile, 1276 loci
SNP-based phylogeny, plasmid and prophage integrated elements

- Plasmid absent from company C
- Evidence of 3 prophage integrated elements
- CJIE1v identified in 3 locations in PacBio reference strains

French et al Emerg Infect Dis. 2019 Dec;25(12):2226-2234
Transmission of *Campylobacter* in the poultry industry

- Within company?
- Local spread?
- Spread through contact networks?
  - Feed
  - Chicks
  - Personnel
Transmission in the poultry industry

Pairwise genetic distance matrix among *C. jejuni* isolates

Geographical distance matrices

Network distance matrices

Strength of association

Farm Covariates

Significant factors

1. Poultry supplier
2. Road distance
3. Network distance of transporting feed vehicles

Greening et al 2020 under review
Comparing genetic relatedness of isolates revealed association with parent company and geographical location (evidence of local spread)
Modelling genetic distance and potential transmission networks

Multivariable model (Distance-based redundancy analysis):
Parent company, farm and road and feed networks associated with genetic distance
Key questions addressed by whole genome sequencing

- How long has it been in NZ?
  - ~mid-late 2013.
- How has it been transmitted between poultry companies?
  - Within company
  - Local spread – road/feed network
- What is the main source of infection?
  - All companies contributing to human infection
- Genetic basis for resistance?
  - megaplasmid and mutation
Whole-Genome Sequencing of a *Campylobacter jejuni* Strain Isolated from Retail Chicken Meat Reveals the Presence of a Megaplasmid with Mu-Like Prophage and Multidrug Resistance Genes

Daya Marasini, Mohamed K. Fakhr
Department of Biological Science, The University of Tulsa, Tulsa, Oklahoma, USA

Genome sequencing of *Campylobacter jejuni* strain T1-21 isolated from retail chicken meat revealed the presence of a chromosome of 1,565,978 bp and a megaplasmid of 82,732 bp that contains Mu-like prophage and multidrug resistance genes. This is the first reported sequence of a *Campylobacter* megaplasmid >55 kb.
Public health significance?

• Resistant to two classes
  – Unless invasive, antibiotics not recommended
  – Sensitive to GP drug of choice, erythromycin
  – ...but others still given by some GPs
• New strain hasn’t caused increase in notifications
• But potential for spread of other, more virulent pathogens

<table>
<thead>
<tr>
<th>Symptom/treatment</th>
<th>Auckland (%)</th>
<th>Manawatu (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital admission</td>
<td>59/445 (13.3)</td>
<td>36/220 (16.4)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>159/442 (36.0)</td>
<td>94/217 (43.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of antibiotics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cipflox</td>
<td>2/147 (1.4)</td>
<td>0/87 (0.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>28/147 (19.1)</td>
<td>11/87 (12.6)</td>
</tr>
<tr>
<td>Ciproxin</td>
<td>2/147 (1.4)</td>
<td>2/87 (2.3)</td>
</tr>
<tr>
<td>E-Mycin</td>
<td>10/147 (6.8)</td>
<td>6/87 (6.9)</td>
</tr>
<tr>
<td>ERA</td>
<td>1/147 (0.7)</td>
<td>0/87 (0.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>53/147 (36.1)</td>
<td>36/87 (41.4)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>3/147 (2.0)</td>
<td>2/87 (2.3)</td>
</tr>
<tr>
<td>Other</td>
<td>48/14 (32.7)</td>
<td>30/87 (34.5)</td>
</tr>
</tbody>
</table>
Source Assigned – case control study design using WGS

Conducted 2018-19
>600 cases, 600 controls
Over 150 isolates each from poultry, cattle, sheep
>1200 isolates sequenced
Risk factors associated with poultry and cattle
Current situation

- ST-6964 was 4\textsuperscript{th} most prevalent in human cases in 2018/19
- Most prevalent in poultry
- Recent work with industry indicates carry-over between flocks important for ST6964 transmission

Source SACNZ study

https://microreact.org/project/ds4r5sfegYQaHg4mzttZCo

Conclusions

• Campylobacteriosis still concerning for New Zealanders despite significant gains in 2006/7
  – Sporadic food-associated cases
  – Large waterborne outbreak in 2016
  – Emergence of AMR linked to poultry consumption
• Poultry industry, government and research providers working together to achieve targets and further drive down contamination and notification rates
• AMR situation can change very rapidly
• MPI new strategy
• Advances in genome sequencing and modelling playing increasingly important roles in determining origin, evolution, transmission and prevention

Requires a One Health approach
Acknowledgements

- mEpiLab team: particularly David Wilkinson, Ji Zhang, Jonathan Marshall, Patrick Biggs, Lynn Rogers and Anne Midwinter

- Carolyn Gates, Sabrina Greening, Jing Liao, Marti Anderson, Petra Mullner and Martin Hazelton

- ESR: Rob Lake, Phil Carter, Brent Gilpin, Kristen Dyet

- University of Melbourne: Debbie Williamson, Ben Howden, Dieter Bulach, Glen Carter, Sarah Baines, Anders Gonçalves da Silva, Danielle Ingle

- University of Otago: Michael Baker

- MidCentral Public Health, MedLab Central

- Ministry for Primary Industries team: Donald Campbell, Steve Hathaway

- Poultry Industry Association