Niche adaptation and resistance progression in *Campylobacter*

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Population genomics and evolution of bacteria

Host - Sheppard et al. (2013) *PNAS*
Biofilm – Pascoe et al. (2015) *Env Micro*
Survival - Yahara et al. (2017) *Env Micro*

Pathogenicity – Monteil et al. (2016) *MGen.*
Pathogenicity – Bayliss et al. *In prep.*
Host/pathogen - Berthenet et al. (2018) *BMC Biology*
*Campylobacter*: among the most common causes of bacterial gastroenteritis

Antimicrobials used in persistent or severe cases but infection is usually self limiting (3-5) days.

Humans are a dead end host so where is the selection for resistance?
C. jejuni and C. coli principally inhabit animal guts

Is AMR acquired in animal hosts?

Sheppard et al. (2009) *Clinical Infectious Diseases* 48:1072–1078
Sheppard et al. (2010) *Applied Environmental Microbiology* 76, 5269-5277
Not all host animals are equal.

>20 billion chickens          ~1.5 billion cattle

Global use of antimicrobials in livestock production:
• Many of the same antimicrobial classes
• Global distribution networks
• Growth promoters and treatment of ‘sick animals’

The rise of AMR in *Campylobacter* from humans and animals coincides

Trends in fluoroquinolone resistance in *Campylobacter*

Putative ciprofloxacin resistance in 1038 human (Blue), 670 chicken (Orange) and 136 ruminant (Grey) samples collected from 1978 to 2017.

Sproston et al. (2018) *Microbial Genomics* 4; doi:10.1099/mgen.0.000198
Understanding the spread of AMR *Campylobacter*

1. Clonal transmission
2. Gene pool transmission
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Campylobacter source attribution studies

Several potential sources.

Different hosts have different *C. jejuni* types so disease can be attributed.
National attribution experiment

Disease isolates
- 5674 screened
- 999 C. jejuni or C. coli positive

Non-clinical isolates*
- 3417 screened

Potential source genotypes
- 5247 genotyped
- 2420 archive genotypes

Model comparison

*Non-clinical isolates are from food, host animal and environmental sources

Sheppard et al. 2009 *Clinical Infectious Diseases* 2009;48:1072–1078
Chicken is a major source of human infection

Assignment of clinical isolates to source using the Bayesian clustering algorithm - STRUCTURE.
Chicken is a major source of human infection

Assignment of clinical isolates to source using the Bayesian clustering algorithm - STRUCTURE.
Data and models have improved

1. Model limitations can be quantified by self attribution

2. Genome-wide host-segregating markers

Berthenet et al. (2019) Scientific Reports 9: 8098
Chicken (followed by ruminants) remain the major source of human campylobacteriosis

Source attribution (C. jejuni)
- 60 – 80 % attributed to chicken
- 40 – 60 % to ruminant
- 4 – 10 % to wild birds & environment

Biology limits:
Zoonotic transmission occurs every 1.6 years in some C. jejuni lineages so association signal is lost.

Berthenet et al. (2019) Scientific Reports 9: 8098
Understanding the spread of AMR *Campylobacter*

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AMR *Campylobacter* from humans, animals and sewage.

<table>
<thead>
<tr>
<th>Antibioticsa</th>
<th>Campylobacter jejuni (n = 162)</th>
<th>Campylobacter coli (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals</td>
<td>Humans</td>
</tr>
<tr>
<td>Total number of non-multidrug resistant</td>
<td>27/44 (61.36%)</td>
<td>101/115 (8.69%)</td>
</tr>
<tr>
<td>Total number of multidrug resistant</td>
<td>17/44 (38.63%)</td>
<td>10/115 (87.82%)</td>
</tr>
<tr>
<td>Total number of isolates</td>
<td>44</td>
<td>115</td>
</tr>
</tbody>
</table>

a Antibiotic resistance to: ciprofloxacin; tetracycline; erythromycin; streptomycin; gentamicin.

Florez-Cuadrado et al (2017) Frontiers in Microbiology 8, 2240

MDR is higher in *C. coli* than in *C. jejuni*

Presence/allelic diversity of 15 AMR genes, based on CARD, ResFinder and NCBI databases.
AMR is distributed across structured *Campylobacter* populations – not just successful clones.
AMR genes in genomic islands

A. Animals

- C. jejuni (Cattle, 1)
  - tetO
  - ant(6)-la
  - sat-4
  - aph(3')-Ila
  - pCJ14980A

- C. jejuni (Chicken, 1)
  - ant(6)-la
  - ant(6)-lb
  - pCFSAN032805

- C. coli (Chicken, 1)
  - aad9
  - hpt

- C. coli (Chicken, 1)
  - invC
  - ΔtetO

- C. coli (Pig, 1)
  - pCFSAN032805

- C. coli (Chicken, 1)
  - aph(2)-Ila

- C. jejuni (Chicken, 3)
  - aprA
  - ermB

B. Humans

- C. coli (2)
  - ant(6)-la
  - sat-4
  - aph(3')-Ila
  - integrative conjugative mobile element

- C. coli (1)
  - hpt
  - pCFSAN032805

- C. coli (1)
  - ΔtetO
  - Δant(6)-la
  - aph(2)-Ila

- C. coli (3)
  - aprA
  - pCFSAN032805, pTet

- C. jejuni (1)
  - pCFSAN032805

C. Sewage

- C. coli (1)
  - ant(6)-la
  - sat-4
  - aph(3')-Ila
  - pCFSAN032805

- C. coli (1)
  - Δant(6)-la
  - aph(2)-Ila

- C. coli (1)
  - ΔtetO

- C. coli (1)
  - hpt

- C. coli (1)
  - hpt

- C. coli (1)
  - pCFSAN032805

- C. coli (3)

Evidence of gene pool transmission in *C. coli* from multiple sources

Co-localized *tetO, aad9, ant(6)-lb*

Evidence of gene pool transmission between *C. coli* and *C. jejuni*

Co-localized *ant(6)-la, sat-4, aph(3)-IIla*

Evidence of gene pool transmission

The mean consistency index (CI) was significantly higher among AMR genes (0.66) compared with core genes (0.46), and allelic variation was lower.

Consistent with HGT facilitating the movement of AMR genes.
Shared alleles among β-lactam, tetracycline and aminoglycoside AMR genes (present in > 5 isolate genomes) are numbered around the perimeter.
Understanding the spread of AMR *Campylobacter*

1. Clonal transmission ✓
2. Gene pool transmission ✓

Is this enough to explain the coincidence of rising AMR?

Not really – overlapping gene pools but no source sink dynamics!
Acknowledgements

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