Isolation and identification of *Campylobacter*

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Campylobacter

- Gram negative bacterium
- 23 species
- Sensitive for heat, dryness, disinfection,
- Many animal species are asymptomatic carrier of Campylobacter
  - C. jejuni (92% of gastro-intestinal infections)
  - C. coli (5% of gastro-intestinal infections)
  - C. lari
  - C. upsaliensis
  - C. fetus (blood cultures - systemic)
Animal diseases and *Campylobacter*

- Many animal species are asymptomatic carrier of *Campylobacter* (and source for human infections)

- Clinical disease in animals:
  - *C. jejuni* (abortion in cattle, sheep, alpaca)
  - *C. fetus* subspecies *venerealis*
  - *C. fetus* subspecies *fetus*
  
  Can cause abortion in cattle, sheep, alpaca
Campylobacter - human disease

- Most common bacterial cause of foodborne disease in Europe; second most common in US

- Common cause of diarrhea in infants and young children in developing countries

- 1999 CDC estimated 2.4 million cases annually in US – 13,000 hospitalizations and 120 deaths

- European Union: 2-20 million cases annually (2009)

- Global burden ?? (missing data)
  - Foodborne Disease Epidemiology Reference Group (FERG): Initiative to Estimate the Global Burden of Foodborne Diseases.
Campylobacteriosis

- Outbreaks are rare or....
- Low infectious dose
- Acute gastro-enteritis (self limiting)
- Incubation time 2-5 days
- Sepsis and extra-intestinal infections are rare
- Post-infection complication: Guillain-Barré Syndrome (0.1%)
Top 10 of food borne pathogens (US)

1. Norwalk like viruses  9,200,000
2. Campylobacter      1,963,000
3. Salmonella (non-typhoid)  1,342,000
4. *Clostridium perfringens*  249,000
5. Giardia lamblia     200,000
6. Staphylococcus      185,000
7. Toxoplasma gondii   112,000
8. VTEC (E. coli)       92,000
9. Shigella            90,000
10. Enterotoxigenic E. coli  56,000
Age-specific incidence of clinical *Campylobacter* infections in industrialized and developing countries
Control of campylobacteriosis

• Epidemiological questions:
  – sources and routes of infection (tracing)
  – host specificity?
  – all strains pathogenic for humans?
  – strains associated with specific disease?
Campylobacteriosis: sources of infection

- Poultry meat
- Contaminated drinking water
- Travelling
- Raw milk
- Direct animal contact
- Cross-contamination
Isolation of thermotolerant or thermophilic *Campylobacter* species

- *C. jejuni*
- *C. coli*
- *C. lari*
- *C. upsaliensis*
- *C. helveticus*
Factors Affecting Growth and Isolation

- Culture media and supplements
- Antimicrobial agents
- *Campylobacter* selective agars
- Passive filtration
- *Campylobacter* enrichment broth
- Temperature of incubation
- Micro-aerobic conditions
### Culture Media Supplements (examples)

<table>
<thead>
<tr>
<th>Defined substrates</th>
<th>Complex substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulphate</td>
<td>Blood</td>
</tr>
<tr>
<td>Sodium metabisulphite</td>
<td>Serum</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>Charcoal</td>
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# Antimicrobial Agents in Campy-media

## Inhibitory to Gram negative organisms
- Cephalosporins*
- Trimethoprim TMP
- Polymyxin B/Colistin
- Novobiocin

## Inhibitory to Gram positive organisms
- Vancomycin / Teicoplanin
- Bacitracin
- Rifampicin*
- Sodium deoxycholate

## Inhibitory to fungi and yeasts
- Cyclohexamide
- Amphotericin

* Activity against both Gram negative and positive organisms
Campylobacter Selective Agars and Methods

• Butzler agar (modified)
• Campy Cefex agar
• Charcoal cefoperazone deoxycholate agar
• Charcoal - Amphoteracin - Teicoplanin agar
• Karmali agar
• Preston agar
• Skirrow agar
Passive Filtration Method (Steel & McDermott 1994)

1. Place the membrane onto a Blood agar
2. Add drops of suspension
3. Incubate at 37°C for >48h
4. Remove the membrane

Campylobacter colonies
Campylobacter Enrichment Broths

Preston broth - 1982

Doyle and Roman broth - 1982

Exeter broth - 1986

Park and Sanders broth - 1989
Temperature of Incubation

- 43°C
- 42°C
- 41.5°C
- 37°C
- 37°C > 42°C
- 32°C > 37°C > 42°C
Microaerobic Requirements of Campylobacters

- 5-10% oxygen (Bolton & Coates 1983)
- 1-10% carbon dioxide (Bolton & Coates 1983)
- 1% oxygen (Henderson et al. 2000)
- 5-10% carbon dioxide (Henderson et al. 2000)
- 5-9% hydrogen (Henderson et al. 2000)
Methods of producing microaerobic conditions

- Candle jars/spirit burner
- Gas generating kits
- Evacuation replacement method
- Variable Atmosphere Incubator

Quality control is important:
Always use control strips and reference strains
Factors Affecting Isolation of Campylobacters

- Type of sample to be cultured
- Number and type of competing organisms
- Number of campylobacters present
- Physiological status of campylobacters
- Culture media and isolation protocol
Isolation of *Campylobacter* from clinical specimens

- **Faecal samples**
  - Direct plating
  - Temperature of incubation
  - Period of incubation
  - Passive filtration
  - Enrichment culture

- **Blood cultures and other clinical specimens**
Enrichment Culture for clinical specimens

• Not with acute phase specimens

• Of benefit if:
  • convalescent phase specimens
  • delayed specimens
  • family contacts (asymptomatic)
  • investigating outbreaks
Types of Samples

- Faecal
- Intestinal contents
- Rectal swabs
- Cloacal swabs
- Carcass swabs
Isolation of Campylobacters from Animals

- Direct plating
- Enrichment culture
- Passive filtration
Isolation from Food and Water Samples

Microbiological Examination of Food and Animal Feeding Stuffs
Part 17. Detection of Thermotolerant Campylobacter

• ISO 10272

• Evaluation of the recommended ISO 10272:2006
• Vs. three enrichment media: Bolton broth, Preston broth and CampyFood broth
• Three selective plating agars: modified charcoal cefoperazone deoxycholate agar (mCCDA), CampyFood agar(CFA) and Brilliance CampyCount agar(BCC)

• Compared to the current ISO method, some alternative combinations of enrichment and agar media could provide significantly better detection and enumeration of Campylobacter in chicken meat.

Still use of the ISO method is → Validated method → improves data quality and comparability between regions, countries and laboratories
Validation of methods or use of validated and well described methods (ISO) is important

• ISO methods are well described and validated methods

• Any method used should be validated in your own lab.

• Implementation of quality control measures is important.

• Training of laboratory personell is important.
Identification of Campylobacter

• Detection of Campylobacter in samples (feces, food, water)
  – present yes/no

• Confirmation of isolated strains
  – Campylobacter yes/no

• Identification
  – what species

Important:
Validation of identification method by use of known strains
Use of reference strains for positive and negative controls
Biochemical tests used in our laboratory

- Campylobacter suspect colonies on e.g. mCCD agar.

Verified as Campylobacter by:
  - Microscopy
  - Oxidase test

- Identification to species level:
  - Catalase test
  - Ability to hydrolyse indoxyl acetate
  - Ability to hydrolyse hipporate (be aware that some C. jejuni can give a weak response and will thereby be taken for C. coli)
PCR-types

• PCR with primers specific for one species

• PCR with primers that amplify several species
  – discrimination between species after digestion of the fragment with a restriction enzyme (PCR-RFLP)

• Multiplex PCR: combination of specific PCR’s in one tube
Species specific PCR tests


- *Arcobacter*: A. cryaerophilus, A. butzleri, A. skirrowii
PCR-RFLP

• Several PCR-RFLP’s described

• for unknown isolates:
  – “CAH-PCR” (Campylobacter, Arcobacter, Helicobacter)
  – if Campy: PCR-RFLP for discrimination of thermophilic Campylobacters
Multiplex PCR

• Combination of 2 PCR’s:
  1. C. jejuni specific
  2. C. coli specific

• Several PCR’s for jejuni and coli are described
  (for overview: see Stephen On)
Results of the PCR

1 = C. jejuni
2 = C. lari
3 = C. jejuni
4 = C. coli

- **C. jejuni**: 773 bp and **C. coli**: 364 bp
- If any other Campylobacter species (or ID failure): no band
- ID failures are often found by microscopy
Summary

• Isolation
  – Methods depends on sample type.
  – Different ways to obtain microaerophilic condition
  – Control growth condition by use of reference strain
  – Validation and quality controls measures of method is important

• Identification to campylobacter or species level:
  – PCR
  – Biochemical test
  – Validation of method with known strains and use of reference stains for quality control
  – Microscopy often reveals ID failures